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INACTIVATION OF HEPATITIS A VIRUS (HAV) BY CHLORINE AND IODINE IN WATER

ANNUAL REPORT

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The inactivation of purified, aggregated hepatitis A virus (HAV), poliovirus 1 and echovirus 1 by 1 and 5 mg/l free chlorine and 1 and 2 tablets per quart Army iodine (globaline) in buffered, demand-free water at pH 4.5, 7.0 and 9.5 and 5° and 25°C was determined. HAV inactivation by chlorine was always rapid (99.99% in <8 min.) Polio 1 and echo 1 inactivation by chlorine was rapid at pH 4.5 and 7.0 (99.99% in< 8 min.) but slower at pH 9.5, especially at 1 mg/l and 5°C (59 to ≫60 min.). Inactivation of all test viruses by iodine was always rapid at pH 9.5 (99.99% in < 9 min.) but relatively slow at pH 4.5. At pH 7.0, iodine inactivation was rapid for all viruses at 25°C, but at 5°C, it was rapid for HAV, slow for echo 1 and moderate for polio 1. Thus, Army iodine may not inactivate all viruses rapidly under some field conditions. Further studies are needed to determine if virus inactivation by free chlorine and Army iodine is reduced by dissolved organics and suspended solids in water. Keywords: Water treatment

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SUMMARY

Batch laboratory experiments were done on the kinetics and extent of inactivation of aggregated preparations of hepatitis A virus (strains HM175 and MD-1), poliovirus 1 (strain LSc) and echovirus 1 (strain V239) by 1 and 5 mg/l free chlorine and 1 and 2 tablets per quart of Army iodine (globaline) in phosphate buffered halogen demand-free water at pH 4.5, 7.0 and 9.5 and temperatures of 5 and 25°C. HAV was rapidly inactivated by free chlorine under all conditions tested, with times for 99.99% inactivation (T-99.99) of <8 minutes. Polio 1 and echo 1 were also inactivated rapidly by free chlorine at pH 4.5 and 7.0, with T-99.99 values of 34 minutes or less. However, polio 1 and echo 1 were inactivated slowly by 1 mg/l free chlorine at pH 9.5 and 5°C.

Inactivation of all three test viruses by Army iodine was rapid at pH 9.5 (T-99.99 <8 minutes) under all conditions tested, but they were inactivated relatively slowly at pH 4.5 and 5°C. At pH 7.0, iodine inactivated HAV rapidly but polio 1 and echo 1 were inactivated slowly, especially at 5°C and the lower dose of 1 tablet per quart. These results indicate that Army iodine may not inactivate HAV and other enteroviruses efficiently under some conditions likely to be encountered in the field. Further studies are needed to determine the efficiency of viral inactivation by free chlorine and Army iodine in lesser quality water containing typical interfering materials such as clay turbidity and dissolved organic matter in the form of humic and fulvic acids.

FORWARD

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I. INTRODUCTION AND BACKGROUND

A. Importance of Hepatitis A Virus in Water

Hepatitis A or infectious hepatitis continues to be an important waterborne viral disease. A recent compilation of reported outbreaks of waterborne disease in the United States listed a total of 23 outbreaks involving 737 cases of hepatitis A for the period 1971-1985 (Craun, 1988). In community water supplies most outbreaks of waterborne infectious disease, including outbreaks of hepatitis A, are caused by post-treatment contamination of finished waters in distribution systems (Lippy and Waltrip, 1984). In small and individual water supplies most outbreaks of hepatitis A and other infectious diseases are caused by lack of, interruption of, or inadequate treatment, including disinfection (Lippy and Waltrip, 1984; Craun, 1988). These epidemiological data demonstrate the importance of adequate treatment, and especially adequate disinfection, to prevent waterborne transmission of hepatitis A and other infectious diseases.

Recent reports suggest that HAV may be more resistant to various chemical and physical agents (Siegl et al., 1984) and more stable under various environmental conditions (Sobsey et al., 1986) than other viruses and bacteria. Conventional water treatment practices utilizing chemical disinfection, primarily chlorination, are generally believed to be effective in producing microbiologically safe drinking water. However, the growing number of reports on the isolation of viruses from treated drinking water (Bitton et al., 1986) suggest that some viruses may survive treatment under certain conditions. The establishment of reliable water treatment practices and water quality standards to insure the virological safety of water supplies can be achieved only by fully understanding the response of HAV to water disinfectants such as chlorine and iodine.

B. Previous Studies on Disinfection of HAV in Water

Despite the need to determine the kinetics and extent of HAV inactivation by water disinfectants, no studies on inactivation of HAV in water have been reported and the few investigations reported to date on HAV inactivation by chlorine have been inadequate due to technical limitations. Early studies by Neefe et al. (1945, 1947) provided indirect evidence that HAV is insensitive to combined chlorine. Using human volunteers for virus infectivity assay, Neefe and co-workers found that a total chlorine residual of 1 mg/l did not completely inactivate HAV in dilute fecal suspensions after a contact time of 30 minutes. The addition of sufficient chlorine to produce total and free chlorine concentrations of 1.1 and 0.4 mg/l, respectively, in purified effluent was required to prevent clinical infectious hepatitis in volunteers. More recently, Peterson et al. (1983)

used marmosets to assay for HAV infectivity after chlorination of a partially purified preparation of HAV. The infectivity of the preparation, which contained about 1500 infectious units/ml, was only partially reduced by treatment with up to 1.5 mg/l of free residual chlorine at neutral pH for 30 minutes. These results, along with observations made during an outbreak of hepatitis A from a chlorinated groundwater supply in Georgetown, Texas (Hejkal et al., 1982), suggest that HAV is more resistant to conventional water chlorination processes than other enteroviruses and indicator bacteria. In contrast, results of studies by Grabow et al. (1983) indicated that HAV may be more sensitive to free chlorine than previous studies and epidemiological evidence have suggested. Using serological techniques for assay of HAV infectivity in cell culture, Grabow and co-workers found that HAV was very sensitive to low levels of free chlorine relative to selected indicator viruses and bacteria. However, other studies by this group indicated that HAV was relatively resistant to combined forms of chlorine (Grabow et al., 1984).

C. Military Need for Virus Disinfection of Water

In the military there is a special need for disinfectants that will be effective in destroying waterborne pathogens under adverse or emergency conditions, particularly when the quality of the water available for consumption is poor. Since World War II, the Armed Forces of this country have relied primarily on globaline tablets, an iodine-based disinfectant, for disinfection of canteen water and other small-volume field water supplies (O'Conner and Kapoor, 1970). Relatively little is known about the adequacy of this disinfectant in preventing the transmission of viral pathogens such as HAV in waters with varied physical and chemical characteristics. Outbreaks of infectious hepatitis associated with military activities have continued to occur since the development of globaline (Bancroft and Lemon, 1984). Although the effectiveness of globaline and other forms of iodine against HAV has not been evaluated, disinfection studies on their effectiveness for other enteric viruses, enteric bacteria and protozoan cysts have been reported (Safe Drinking Water Committee, 1980). However, given the substantial differences in the response of different enteric viruses to chlorine and other disinfectants (Liu et al., 1971; Sobsey, 1988) it is impossible to predict the efficacy of globaline or other disinfectants for inactivation of HAV in water.

In view of the limited data on HAV disinfection in general and the inconsistent findings of the few studies on HAV disinfection by chlorine, a critical evaluation of HAV inactivation by free and combined forms of chlorine and by iodine (globaline) is clearly warranted. Water quality variables, such as the presence of suspended inorganic particulates and soluble and colloidal organic matter, are important factors that need to be

evaluated for their effect on the efficiency of disinfection of HAV. The adsorption of viruses to particulate matter in water has been well documented (Goyal and Gerba, 1979; Hurst et al., 1980; Schaub and Sagik, 1975; Sobsey et al., 1980). Particulates may protect viruses in the aqueous environment by sheltering viruses from disinfectant exposure or by consuming or chemically changing the disinfectant. Naturally-occurring soluble and colloidal organic matter (such as humic and fulvic acids) in natural waters, finished waters and wastewaters may also be a factor in reducing the efficacy of the disinfection process by consuming or changing the active species of the chemical agent. The effects of suspended matter and soluble and colloidal organic matter on HAV disinfection by chlorine and iodine have not been adequately addressed in the recent studies reported in the literature.

The study of HAV inactivation kinetics by chlorine and iodine is now feasible with the utilization of new methodologies for the cultivation and enumeration of HAV in cell cultures (Daemer et al., 1981; Frosner et al., 1979; Lemon et al., 1983; Provost and Hilleman, 1979). The focus of this project is to examine the kinetics and extent of HAV inactivation by chlorine and iodine, with special emphasis on determining the influence of important water quality variables on chlorine and iodine inactivation of HAV and, for comparison, selected model viruses. This report covers research progress during the second year of the project, and, for clarity and completeness, it also includes data from experiments done during the first year of the project.

II. STATEMENT OF THE PROBLEM AND OBJECTIVES

A. Statement of the Problem

The problem to be studied is the efficiency (kinetics and extent) of inactivation of hepatitis A virus (HAV) by chlorine (calcium hypochlorite) and Army iodine (globaline) in waters of different quality. In these experiments the inactivation of other viruses or of different strains of HAV is compared.

B. Specific Ojectives

The specific objectives of this study are to determine the kinetics and extent of HAV inactivation by free chlorine (hypochlorite ion/hypochlorous acid) and by iodine (globaline tablets) in waters of different quality. As an alternative to using highly purified, monodispersed virus preparations, disinfection experiments utilize partially purified, aggregated preparations in order to better model the physical state of the viruses in natural aquatic environments.

The water quality variables to be studied are water type, pH and temperature because all of these factors greatly influence the

effectiveness of microbial inactivation by chemical disinfectants (Sobsey, 1988). The two water qualities are buffered halogen demand-free (BHDF) water and so-called worst case water consisting of BHDF water containing 5 NTU of bentonite clay turbidity and 10 mg/l of a 1:1 (W/W) mixture of humic and fulvic acids. Worst case water contains both particulates (in the form of bentonite clay at a turbidity of 5 NTU) and soluble and colloidal organics (in the form of 1:1 mixtures of humic and fulvic acids at a concentration of 10 mg/l) because these are major water quality factors influencing the effectiveness of microbial inactivation by chemical disinfectants. The pH levels to be studied are pH 4.5, 7.0 and 9.5, because this represents the range of pH levels likely to be encountered in drinking waters. The temperatures to be studied are 5° and 25°C, which covers most of the temperature range that may be found in drinking waters.

Waters are seeded with a mixed virus preparation containing sufficient quantities of HAV, poliovirus 1, and echovirus 1 to follow the inactivation of each virus over at least 4 $\log_{10}$ units (99.99%). The latter two viruses are included in order to compare HAV sensitivity to halogen disinfectants to other viruses which have been previously studied in this regard.

It should be noted that some of the objectives of this study have been modified from those originally planned due to technical limitations, constraints arising from limitations of resources, and redundancy to other experiments. Specifically, experiments using 3.6 mg/l iodine, groundwater only, clay turbidity only, organic acids only, MS2 bacteriophage and Escherichia coli have been eliminated.

III. METHODS AND MATERIALS

A. Viruses, Cell Cultures and Virus Purification

1. HAV. The HM175 (NIH prototype) strain of HAV, originally isolated from feces of an infected human in Australia (Daemer et al., 1981; Lemon et al., 1983; Gust et al., 1985) was produced in persistently infected BS-C-1 cells grown in 850 cm^2 roller bottles or 6000 cm^2, ten-tiered cell factories (Inter Med, A/S NUNC, Roskilde, Denmark) incubated at 37°C. Prior to persistent infection, the virus had been serially passaged 6 times in marmosets, 10 times in primary African green monkey kidney (AGMK) cells and 7 times in BS-C-1 cells. The MD-1 strain of HAV, originally isolated from contaminated groundwater implicated in a community-wide outbreak of hepatitis A (Sobsey et al., 1984), was produced in persistently infected A549 (human lung carcinoma) cells grown in roller bottles. This strain of HAV was originally passed 3 times in secondary African green monkey kidney cells prior to passage in A549 cells.

HAV infectivity was assayed by radioimmunofocus assay (RIFA) in BS-C-1 (for strain HM175) or A549 (for strain MD-1) cells as previously described (Lemon et al., 1983; Sobsey et al., 1985), except the incubation period for HM175 in BS-C-1 cells was reduced from about two weeks to one week. The RIFA is an enumerative assay analogous to a plaque assay, except that non-cytopathic, focal areas of infected cells are visualized by an immune autoradiographic method.

Persistently infected cells were passaged every two to four weeks by trypsinization and then resuspension of some of the cells in growth medium at a concentration of about 1×10^5 cells/ml for re-inoculation into culture vessels. At each passage, some of the persistently infected cells and all of the culture fluids were harvested as crude virus stock. Harvested infected cells were centrifuged at low speed (about 3000 x g), resuspended in small volumes of phosphate-buffered saline (PBS), pH 7.5, and extracted with an equal volume of chloroform. The HAV-containing PBS was recovered by low speed centrifugation to remove cell debris and chloroform. The cell debris and chloroform was extracted four to six more times with equal volumes of PBS to obtain additional virus, and all PBS extracts were pooled as virus stock. HAV in culture fluids was concentrated by precipitation with polyethylene glycol (PEG) 6000 (12% w/v, pH 7.2) overnight at 4°C. Resulting precipitates were recovered by low speed centrifugation, resuspended in a small volume of PBS and extracted with a volume of chloroform equal to the PBS volume in order to remove excess PEG. The PBS extracts were cleared of chloroform and PEG by low speed centrifugation.

PBS extracts of cells and PEG concentrates from culture fluids were pooled, and HAV was pelleted by ultracentrifugation at 30,000 RPM (105,000 x g) for 4 hours at 5°C. Resulting pellets were resuspended in small volumes of 0.05M phosphate-buffered distilled water (PBDW) and supplemented with CsCl to give a density of 1.33 g/ml. These samples were ultracentrifuged to equilibrium in self-generated gradients at 25,000 RPM (90,000 x g) and 5°C for 3 days using the SW27 rotor (Beckman Instruments). Gradients were harvested in fractions from the bottoms of the tubes and assayed for HAV infectivity by RIFA. Fractions with the peak of HAV infectivity were desalted by untrafiltration and washing with PBDW using Centricon 30 tubes (Amicon Inc). Desalted fractions were layered onto 10-30% sucrose gradients in phosphate buffered halogen demand-free water, pH 7.5, (PBHDFW) and subjected to rate zonal centrifugation in the SW27 rotor at 25,000 RPM (90,000 x g) and 5°C for 5.5 hours. Under these conditions, single virions would sediment about 2/3rds of the distance from the top to the bottom of the tube. Gradient fractions were harvested from the top of the tube and assayed for HAV infectivity by RIFA. Gradient fractions were characterized as containing single virions or small, medium or large aggregates of HAV according to their

position in the gradient. HAV gradient fractions were then pooled and in most experiments mixed with appropriate amounts of gradient fractions of the other test viruses such that the total amount of each virus consisted of about 8% single virions, 19% small aggregates, 39% medium aggregates and 34% large aggregates. The titer of each virus in the mixture was 1-5 x 10^8 infectious units/ml. For experiments on the inactivation of different strains of HAV by chlorine and iodine, pools of HAV gradient fractions containing the previously specified distribution of singles and different sized aggregates with titers of 1-5 x 10^8 infectious units/ml were tested directly. These virus stocks and mixtures were further diluted 1:5 in halogen demand-free water for use in disinfection experiments in order to reduce halogen demand.

2. Echovirus 1 and Poliovirus 1. Echovirus 1 (strain V239) and poliovirus 1 (strain LSc) were grown and assayed by the plaque technique in BGM (African green monkey kidney-derived) and MA104 (rhesus monkey kidney-derived) continuous cell lines, respectively, as previously described (Sobsey et al, 1978). In order to assay each animal virus type (HAV, poliovirus and echovirus) in samples containing all three viruses, the other two viruses were neutralized by adding antibodies (antisera) against them to the virus diluent. Antisera were reference reagents prepared for the National Institutes of Health, and they were obtained from the American Type Culture Collection, Rockville, Maryland. For example, poliovirus was assayed by neutralizing echovirus type 1 using antiserum against echovirus type 1 in the poliovirus diluent. HAV did not have to be neutralized in assays for poliovirus or echovirus because it was non-cytopathic, grew slowly and did not interfere with the assays for these other two viruses (unpublished results).

Poliovirus and echovirus were first plaque-purified 2-3 times and then grown in large quantities under either one-step growth conditions (>5 PFU/cell) or at low multiplicity of infection (MOI; 0.01-0.1 PFU per cell). Crude virus stocks were harvested from infected cells at 5-7 hours post-infection under one-step growth conditions or from infected cell lysates several days post-infection at low MOI when cytopathic effects were 4+. Virus was liberated from cells and cell debris by freezing and thawing, and then cell debris was removed by centrifugation at low speed (10,000 x g for 15-30 minutes). Viruses in resulting supernatants were pelleted by ultracentrifugation (105,000 x g and 5°C for 4 hours). Resulting virus pellets were resuspended in buffered HDFW, homogenized 1 minute at top speed in an Omni Mixer (Omni International, Waterbury, Connecticut), and in some cases centrifuged at 10,000 x g and 5°C for 20 minutes to remove additional debris. After supplementing the sample with CsCl to give a density of 1.33 g/ml, viruses were banded to equilibrium as for HAV. Gradient fractions were harvested and assayed for virus infectivity, and virus peak fractions were desalted using

Centricon 30 ultrafiltration units. These fractions were pooled and subjected to rate-zonal centrifugation in 5% (or 10%) to 30% sucrose gradients as for HAV. Gradient fractions were harvested and assayed for virus infectivity and appropriate amounts of virus fractions were added to HAV samples to give the desired distribution and virus titers of single virions as well as small, medium and large aggregates.

B. Glassware and Halogen Reagents.

All glassware for disinfection experiments and preparation of halogen demand-free (HDF) virus stocks was soaked at least 4 hours in a strong chlorine (10-50 mg/l) solution and then rinsed thoroughly with halogen demand-free water (HDFW) prior to use. HDFW and buffer solutions for disinfection experiments were prepared from glass-distilled, deionized water by adding chlorine to approximately 10 mg/l. After storage at room temperature for at least 1/2 day, water or buffers were dechlorinated by exposure to a submersible ultraviolet light. HDF, phosphate-based buffers, 0.01M, were used to prepare chlorine test solutions and buffered water for disinfection experiments.

Reagent grade calcium hypochlorite was used to prepare solutions of hypochlorous acid (HOCl) at pH 4.5, predominantly hypochlorite ion (OCl^-) at pH 9.5, and mixtures of these free chlorine species at pH 7.0. Hypochlorite stock solutions of about 100 mg/l were prepared by dissolving about 0.2 g of $Ca(OCl)_2$ in 1 liter of HDFW. Stock solution was then diluted in test water (halogen demand-free, 0.01M phosphate buffer, pH 4.5, 7.0 or 9.5 in initial experiments) to give the target chlorine concentration. Target chlorine concentration was verified by chemical analysis. Iodine solutions were prepared from globaline tablets, which contain tetraglycine hydroperiodide as the active ingredient and disodium dihydrogen pyrophosphate as a buffer. Globaline concentrations to be tested were the two concentrations based upon recommended Army field use: 1 to 2 tablets per quart, giving concentrations of about 8 and 16 mg of titratable iodine per liter, respectively. Iodine solutions were prepared by dissolving 1 or 2 globaline tablets in about 900 ml of HDFW and adjusting to pH 4.5, 7.0 or 9.5 with NaOH or H_2SO_4. These samples were brought to a volume of 927 ml with HDFW and iodine concentration was measured. The volume of iodine solution was such that addition of 1 part of test virus mixture to 9 parts of iodine solution would dilute the iodine to the target concentration. To be used for experiments, samples with 1 or 2 tablets per quart had to have initially measured iodine concentrations of >7.5 and >15 mg/l, respectively. For experiments using the equivalent of 1 tablet per quart, initially measured iodine concentrations averaged 8.2 mg/l for experiments at 5°C and 7.74 mg/l for experiments at 25°C. After 30-60 minutes of contact time, the reaction mixtures (iodine

plus viruses) contained an average iodine residual of 6.0 mg/l for experiments at 5°C and 5.2 mg/l for experiments at 25°C. Iodine losses during the reaction period were greatest at pH 9.5 and least at pH 4.5. At pH 9.5, the HOI formed by hydrolysis of I_2 probably decomposed to iodate and iodide, as previously reported by Chang (1958). Overall, the iodine residuals obtained in this study are generally consistent with those of previous studies. For example, Farrah (1986) used one or two globaline tablets per quart in studies on the inactivation of three enteroviruses and bacteriophage MS2. At one tablet per quart, his initial iodine concentrations at pH 5.0 ranged from 6.7 to 7.2 mg/l, and after 60 minutes they ranged from 7.0-7.1 mg/l. Although the specifications and testing protocol for iodine tablets indicates somewhat higher iodine concentrations than reported here, the test protocol and the analytical methods differ from those used in this study. Therefore, it could be expected that differences in measured iodine residuals could occur. Furthermore, this study was done using a single lot of Army approved globaline tablets. These tablets could have been somewhat deficient in potency or they could have lost potency during continued storage and use. Since the iodine tablets met target iodine concentrations when tested at the re-test date, they were considered acceptable for use and representative of tablets available for actual field use.

C. Halogen Analysis.

Iodine and chlorine concentrations were measured by DPD colorimetric methods as described in Standard Methods for the Examination of Water and Wastewater, 16th edition (American Public Health Association, 1985). Standardization of procedures for chlorine measurement was by the DPD ferrous titration method, and for iodine measurement by using potassium bi-iodate as a primary standard.

D. Protocols for Disinfection Experiments.

Initial experiments on HAV disinfection by free chlorine or Army iodine in buffered HDFW, pH 4.5, 7.0 and 9.5, were done according to the flow diagram shown in Figure 1. Samples were in 16 mm diameter x 100 mm long test tubes placed in a water bath to maintain a temperature of 5 or 25°C. For initial experiments with free chlorine at concentrations of 1 and 5 mg/l or iodine at doses of 1 and 2 tablets per quart (initial iodine concentrations of >7.5 mg/l and >15 mg/l, respectively), 0.85 ml of purified HAV strain (HM175 or MD-1) or virus stock mixture (HAV, polio and echo), diluted 1:5 in HDFW, was added to 7.65 ml of a chlorine solution containing 1.1 or 5.5 mg/l free chlorine or >7.5 or >15 mg/l iodine and briefly mixed. A second test tube containing only chlorine or iodine solution served as a halogen control. A third tube containing a 1:10 dilution of stock virus in buffered, HDFW served as a virus control. Samples of 0.7 ml were withdrawn from the reaction tube (halogen

solution plus added virus) for viral analysis at 0.33, 1.0, 3.0, 10, 30 and 60 minutes after virus addition. These samples were diluted two-fold immediately in virus diluent (2X Eagle's MEM) containing 1% $Na_2S_2O_3$. A further five-fold dilution (10-fold overall) was made, followed by serial 10-fold dilutions made in separate diluents for each virus. These dilutions were stored at 4°C for subsequent virus assay. After the 60 minute reaction period, the remaining reaction mixture (halogen plus added virus) and the halogen control sample (halogen only) were re-analyzed for free and combined chlorine or iodine. In later experiments, halogen residuals were also measured after only 30 minutes of reaction as well. Samples from the virus control tube (virus plus buffered HDFW) were diluted serially 10-fold at the beginning and the end of the 60 minute reaction period for subsequent virus assay.

E. Experiments on Halogen Stability in Mock Samples

In order to better characterize the rate and extent of halogen loss over time in test samples, a series of experiments were done in which 9 parts of halogen solution at 1.1 times the desired concentration was mixed with 1 part of a "mock" purified virus preparation consisting of 23% sucrose in 0.01M phosphate buffered HDF water. Samples of the mixture were analyzed for residual halogen using the methods described above at various times over a 60 minute period. Experiments were done using iodine at a dose of 1 tablet per quart, chlorine at 1 and 5 mg/l, pH levels 4.5, 7.0 and 9.5, and temperatures of 5 and 25°C.

VIRUS CONTROL	TEST SAMPLE	HALOGEN CONTROL
Buffered test Water (2.25 ml) + Viruses (0.25 ml)	Buffered Halogen Solution 7.65 ml) + Viruses (0.85 ml)	Buffered Halogen Solution (10.53 ml) + Diluent (1.17 ml)
Vortex Mix	Vortex Mix	Vortex Mix
Sample Viruses at 0 and 60 min.	Sample Viruses at 20 sec., 1, 3, 10, 30 and 60 min.	

To Sample at each Sampling Time:

0.7 ml Virus Sample
+ 0.7 ml 1.0% $Na_2S_2O_3$
in Virus Diluent

Subsequent Sample Dilutions in Diluent for each Virus (5- then Serially 10-fold)

Determine Halogen Residual in Test Sample after 30 & 60 minutes and in Halogen Control after 60 Minutes

Assay for HAV, Polio 1 & Echo 1 in Appropriate Host Cells

FIGURE 1. FLOW DIAGRAM OF PROTOCOL FOR HALOGEN DISINFECTION EXPERIMENTS

IV. RESULTS AND DISCUSSION

A. Disinfection of HAV, Poliovirus 1 and Echovirus 1 by Free Chlorine in Buffered HDFW.

The mean results of duplicate experiments on inactivation of HAV, poliovirus 1 and echovirus 1 by 1 and 5 mg/l doses of free chlorine at 5 and 25°C and pH 4.5, 7.0 and 9.5 are summarized in Tables 1 and 2 as times for 99.99% virus inactivation (T-99.99) and in Figures 2-13 where log N_t/N_o is plotted versus contact time. The results of individual experiments are given in Appendix Tables A1-A12. At a chlorine dose of 1 mg/l, HAV was inactivated rapidly at all pH levels tested, with T-99.99 values of <8 minutes. As expected, HAV inactivation was more rapid at 25°C than at 5°C, and in most cases the inactivation rate was 2-3 times faster at the higher temperature. It is interesting to note that the rate of HAV inactivation was most rapid at pH 7.0 but not much longer at pH 9.5 than at pH 4.5. This suggests that HAV is either relatively sensitive to hypochlorite ion, the predominant chlorine species at pH 9.5, or that it is highly sensitive to the very low concentration of hypochlorous acid that is present at pH 9.5, or both.

Compared to HAV, both poliovirus 1 and echovirus 1 were more resistant to free chlorine, at least at pH 7.0 and 9.5. In general, echo 1 was inactivated more slowly than polio 1. Both polio 1 and echo 1 were inactivated slowly by a 1 mg/l dose of free chlorine at pH 9.5 and 5°C, with T-99.99 values of 59 and >>60 minutes, respectively. These results are consistent with those of previous studies showing that poliovirus and echovirus are inactivated more slowly at higher pH levels where hypochlorite ion predominates (Engelbrecht et al., 1980; Scarpino, 1979). At 25°C and pH 9.5, inactivation rates of both polio 1 and echo 1 were considerably faster than at 5°C (T-99.99 = 8.4 and 32 minutes, respectively). Both polio 1 and echo 1 were inactivated relatively rapidly at pH 7.0 and 4.5 where greater proportions of the more virucidal hypochlorous acid are present than at pH 9.5. At these pH levels, T-99.99 values for polio 1 and echo 1 were <8 minutes. As observed for HAV, inactivation of polio 1 and echo 1 was consistently greater at 25 than at 5°C.

Data for HAV, polio 1 and echo 1 inactivation by a 5 mg/l dose of free chlorine at 5 and 25°C are shown in Figures 8-13 and summarized in Table 2 as T-99.99 values. HAV was inactivated rapidly by a 5 mg/l dose of free chlorine, with T-99.99 values <3 minutes, and HAV inactivation was greater at 25 than at 5°C. As previously noted for a 1 mg/l dose of free chlorine, HAV inactivation by a 5 mg/l dose of free chlorine was more rapid at pH 7.0 than at pH 4.5 or 9.5, and HAV inactivation times at pH 9.5 were not much longer than those at lower pH levels.

At a 5 mg/l dose of free chlorine and pH 9.5, both polio 1 and echo 1 were inactivated more slowly than HAV. However, at pH 4.5 and 7.0, all three viruses were inactivated very rapidly, with T-99.99 values of <3 minutes. Comparison of T-99.99 values of Tables 1 and 2 indicates that all three viruses were generally inactivated more rapidly by 5 mg/l free chlorine than by 1 mg/l free chlorine, as would be expected. A 5 mg/l free chlorine concentration and 30 minute contact time are Army standard operating conditions for chlorine disinfection of field water supplies. Therefore, it is important to note that HAV and the other two enteroviruses tested (polio 1 and echo 1) were substantially (>99.99%) inactivated under these conditions. These results suggest that enteroviruses will be adequately controlled by present Army conditions for chlorine disinfection of water, at least in the absence of excessive turbidity or other interferences.

B. Inactivation of Different Strains of HAV by Free Chlorine

Because the experiments described above were done only with the HM175 strain of HAV, it was of interest to determine if other strains of HAV would display comparable sensitivity to free chlorine. In these experiments echovirus 1 was included with HAV strain MD-1 to act as an internal control and to verify that its inactivation kinetics were similar to those obtained in previous experiments. The results of experiments on inactivation of HAV strain MD-1 by a 1 mg/l dose of free chlorine at pH 9.5 and 5°C are shown in Figure 14 and in Table 3 as T-99.99 values. The data of these experiments are also given in Appendix Table A13. These results show that the MD-1 strain of HAV was inactivated relatively rapidly (T-99.99 = 5.1 minutes) by a 1 mg/l dose of free chlorine and somewhat more rapidly than strain HM175 (T-99.99 = 7.3 minutes). As in previous experiments, echo 1 was inactivated relatively slowly by a 1 mg/l dose of free chlorine at pH 9.5 and 5°C, with a T-99.99 value of >>60 minutes. The results of these experiments indicate that two strains of HAV, HM175 and MD-1, are relatively sensitive to free chlorine and that their sensitivities are similar.

C. Inactivation of HAV, Poliovirus 1 and Echovirus 1 by Iodine in Buffered HDFW

The mean results of duplicate experiments on inactivation of HAV, polio 1 and echo 1 by Army iodine (globaline tablets) at doses of 1 and 2 tablets per quart, pH 4.5, 7.0 and 9.5, and temperatures of 5 and 25°C are summarized in Tables 4 and 5, respectively, as times for 99.99% virus inactivation (T-99.99) and in Figures 15 to 26 where virus survival as log N_t/N_o is plotted versus contact time. The results are also presented in Appendix Tables A14-A25. These results indicate that HAV is inactivated rapidly by 1 or 2 tablets of iodine per quart at pH 7.0 and 9.5, with T-99.99

values of <2 minutes. At pH 4.5, the pH at which the iodine tablets are buffered, HAV inactivation rates are slower, with T-99.99 values ranging from 7.1 to 61 minutes, depending upon iodine dosage and temperature. As expected, HAV inactivation by iodine was greater at the higher temperature of 25°C than at 5°C. It should be noted that HAV inactivation rates were somewhat slower at the iodine dose of 2 tablets per quart than at the dose of 1 tablet per quart, at least at pH 4.5. The reasons for these somewhat aberrant results are uncertain. It is unlikely that they are due to failures to achieve target iodine concentration at pH 4.5 because 30- to 60-minute iodine residuals averaged 6.46 mg/l at a dose of 1 tablet per quart and 15.2 mg/l at a dose of 2 tablets per quart. Experiments at the two different iodine doses were often done using different virus preparations and different batches of iodinated water. Differenecs in the characteristics of the virus preparations, such as the size distribution of virus aggregates, or in the characteristics of the iodine solutions, such as the proportions of different iodine species in solution, could have contributed to differences in virus inactivation rates.

Results in Tables 4 and 5 and Figures 15 to 26 for inactivation of polio 1 and echo 1 by 1 and 2 iodine tablets per quart indicate that both viruses were inactivated relatively rapidly at pH 7.0 and 9.5 and a temperature of 25°C, with T-99.99 values of <8 minutes. Inactivation times for polio 1 and echo 1 under these conditions were somewhat longer than those for HAV in most cases. Inactivation of polio 1 and echo 1 at pH 9.5 and 5°C was also relatively rapid, with T-99.99 values of 15 minutes or less. Again, inactivation of these two viruses was slower than that of HAV under corresponding conditions. Inactivation of polio 1 and echo 1 was quite slow at pH 4.5 and 5°C, with T-99.99 values ranging from 58 to >>60 minutes. Inactivation of polio 1 by both iodine doses was also slow at pH 7.0 and 5°C (T-99.99 >>60 minutes). At pH 4.5 and 25°C, inactivation of polio 1 and echo 1 was faster than that at the same pH and 5°C, with T-99.99 values ranging from 35.7 to 11.4 minutes. At 25°C and pH 7.0, inactivation of polio 1 and echo 1 was quite rapid, with T-99.99 values ranging from 1.1 to 4.3 minutes.

Overall, the results of these studies indicate that all 3 viruses were inactivated rapidly by iodine doses of 1 and 2 tablets per quart at pH 9.5. However, at the lower pH values of 7.0 and especially 4.5 and the lower temperature of 5°C, inactivation rates of at least some test viruses, especially polio 1, were quite slow. These findings have important implications for the current formulation and use of Army iodine because neither 1 nor 2 tablets per quart will substantally inactivate some enteric viruses in a short time period at the acid pH levels the tablets are intended to achieve.

The finding that HAV is less resistant or similar in resistance to iodine inactivation than are the other two test viruses is

generally consistent with the results obtained using free chlorine. However, some differences can be noted between chlorine and iodine. At pH 9.5, all three viruses are rapidly inactivated by iodine doses of 1 and 2 tablets per quart. In contrast, HAV is inactivated relatively rapidly at pH 9.5 by 1 and 5 mg/l free chlorine but polio 1 and echo 1 are inactivated quite slowly, especially at 5°C. At pH 7.0, 1 and 5 mg/l free chlorine rapidly inactivates all three viruses (T99.99 <4 minutes), but iodine rapidly and consistently inactivates only HAV at pH 7.0 and both temperatures. At pH 4.5, chlorine rapidly inactivates all 3 viruses (T-99.99 <8 minutes), but iodine inactivates them relatively slowly, especially at 5°C. These results demonstrate clear differences in the effectiveness of chlorine and iodine for virus inactivation at different pH levels, and they highlight the importance of maintaining optimum pH to achieve efficient virus inactivation.

D. Stability of Free Chlorine and Iodine in Mock Experimental Water

Because of concerns about the stability of halogens in test samples, mock virus inactivation experiments were done in which sucrose in PBDFW (mock virus stock) was added to 1 and 5 mg/l chlorine solutions and 1 tablet per quart iodine solutions in PBDFW at pH 4.5, 7.0 and 9.5 and 5 and 25°C. These solutions were sampled at various times over 60 minutes and analyzed for residual free chlorine or iodine. As shown by the results for free chlorine experiments in Figures 27-29, free chlorine was quite stable under most of the conditions tested, with losses of <10% of the initial residual in 60 minutes. Free chlorine losses were somewhat greater at 25°C than at 5°C. Overall, free chlorine was relatively stable in mock experimental waters. These results suggest that the somewhat greater losses of free chlorine observed in some virus inactivation experiments were probably due to additional chlorine demand by the stock virus preparations used.

The results of experiments on iodine stability, which are summarized in Figures 30 and 31, indicate that iodine was quite stable at pH 4.5 and 7.0, with <10% loss of the initial iodine concentration after 60 minutes. Iodine losses were somewhat greater at 25°C than at 5°C. At pH 9.5, however, iodine losses were more extensive, with an approximate 2 mg/l loss in 60 minutes at 5°C and an approximate 4.5 mg/l loss in 60 minutes at 25°C. The results of these iodine stability experiments are generally consistent with those of actual virus inactivation experiments. In virus inactivation experiments iodine losses by 60 minutes averaged 13.5% of the initial iodine at pH 4.5 and 7.0 and 59% of the initial iodine at pH 9.5. Although these results indicate that iodine is relatively unstable at pH 9.5, as has been previously shown (Chang, 1958), there was still sufficient iodine residual to achieve substantial (>99.99%) virus inactivation in 15 minutes or less.

V. SUMMARY AND CONCLUSIONS

An important conclusion that can be drawn from the results of virus inactivation experiments using doses of 1 and 5 mg/l free chlorine in buffered HDFW at pH 4.5, 7.0 and 9.5 and at 5 and 25°C is that all three test viruses are inactivated extensively (>99.99%) by a 5 mg/l dose of free chlorine in 34 minutes or less. In general, virus inactivation was more rapid at the higher temperature and lower pH levels. In addition, the inactivation of HAV was greater than or similar to the inactivation of polio 1 and echo 1 under nearly all conditions tested. These results suggest that the Army disinfection criterion of a 5 mg/l free chlorine residual after 30 minutes for Army field water purification units (reverse osmosis) is sufficient to achieve substantial (>99.99%) inactivation of HAV and other enteroviruses. The results obtained for HAV in this study are generally consistent with those of Grabow et al. (1983), who reported that HAV was relatively sensitive to free chlorine, and they are in contrast to the results of Peterson et al. (1983), which suggested that HAV was relatively resistant to free chlorine.

The observation of greater or equal inactivation of HAV than of polio 1 and echo 1 was also observed using recommended iodine doses of 1 and 2 tablets per quart at pH 7.0 and 9.5 and temperatures of 5 and 25°C. Thus, it appears that HAV is relatively sensitive to inactivation by free chlorine and Army iodine. However, inactivation of HAV, polio 1 and echo 1 by Army iodine was not rapid under all conditions tested, including some conditions at which iodine is typically used. In particular, HAV, polio 1 and echo 1 were not always inactivated rapidly (within 10 minutes) or extensively (>99.99%) at pH 4.5 and 7.0, especially at the lower dose of 1 tablet per quart and the lower temperature of 5°C. These findings are especially important because Army iodine tablets are buffered at low pH in order to minimize the hydrolysis of elemental iodine and to maximize the inactivation of Endamoeba histolytica (Chang, 1958; Morris et al., 1953). The results for HAV as well as those for polio 1 and echo 1 indicate that Army iodine may not efficiently inactivate many enteric viruses under typical field conditions. Furthermore, all experiments reported herein were done in highly purified water that was free of such common interfering materials as dissolved organic matter and turbidity (particulates). These interferences could further reduce the effectiveness of both chlorine and iodine for inactivation of enteric viruses. Additional experiments will be done using water containing interferences and having greater halogen demand than the waters used in the experiments reported above. Such studies are needed before definitive conclusions can be drawn about the relative resistance or sensitivity of HAV and other enteroviruses to different forms of chlorine or iodine in water.

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Table 1. Time for 99.99% Inactivation of HAV, Polio 1 and Echo 1 by 1 mg/l Free Chlorine in PBDFW at pH 4.5, 7.0 and 9.5; 5°C and 25°C*

Virus	Time (Minutes) for 99.99% Inactivation at: pH 4.5	pH 7.0	pH 9.5
		5°C	
HAV	5.2	0.7	7.3
Polio 1	4.2	1.2	59
Echo 1	7.1	3.6	>>60
		25°C	
HAV	2.6	<0.33**	2.2
Polio 1	0.8	0.4	8.4
Echo 1	0.8	1.4	32

*Times for 99.99% inactivation estimated by linear regression analysis of $\log_{10}$ virus survival versus time; mean results of duplicate experiments for each condition.

**Less than (<) values are based on the detection limit for viruses in samples assayed; in cases where one replicate trial gave a measurable virus titer and the other replicate trial was indeterminate, the determinate value was averaged with the detection limit value.

Table 2. Time for 99.99% Inactivation of HAV, Polio 1 and Echo 1 by 5 mg/l Free Chlorine in PBDFW at pH 4.5, 7.0 and 9.5; 5°C and 25°C*

Virus	Time (Minutes) for 99.99% Inactivation at: pH 4.5	pH 7.0	pH 9.5
		5°C	
HAV	2.3	1.0	2.7
Polio 1	0.5	1.1	5.1
Echo 1	0.4	0.6	34
		25°C	
HAV	1.1	0.5	1.0
Polio 1	0.4	0.2	4.4
Echo 1	0.5	0.4	11

*Times for 99.99% inactivation estimated by linear regression analysis of $\log_{10}$ virus survival versus time; mean results of duplicate experiments for each condition.

**Less than (<) values are based on the detection limit for viruses in samples assayed; in cases where one replicate trial gave a measurable virus titer and the other replicate trial was indeterminate, the determinate value was averaged with the detection limit value.

Table 3. Inactivation of HAV, Strains HM175 and MD-1, and Echo 1 by 1 mg/l Free Chlorine in PBDFW, pH 9.5 and 5°C

Virus	Minutes for 99.99% Inactivation
HAV, MD-1	5.1
Echo 1	>>60
HAV, HM175*	7.3
Echo 1*	>>60

*Data from previous experiments (Table 1).

Table 4. Time for 99.99% Inactivation of HAV, Polio 1 and Echo 1 by 1 Tablet/Quart Iodine in PBDFW at pH 4.5, 7.0 and 9.5; 5°C and 25°C*

Virus	Time (Minutes) for 99.99% Inactivation at: pH 4.5	pH 7.0	pH 9.5
		5°C	
HAV	<35**	<1.35	<0.37
Polio 1	>>60	>>60	<4.9
Echo 1	>60	<34	2.9
		25°C	
HAV	7.1	<0.42	<0.30
Polio 1	<36**	<3.5	<7.2
Echo 1	<11	<4.3	<1.4

*Times for 99.99% inactivation estimated by linear regression analysis of $\log_{10}$ virus survival versus time; mean results of duplicate experiments for each condition.

**Less than (<) values are based on the detection limit for viruses in samples assayed; in cases where one replicate trial gave a measurable virus titer and the other replicate trial was indeterminate, the determinate value was averaged with the detection limit value.

Table 5. Time for 99.99% Inactivation of HAV, Polio 1 and Echo 1 by 2 Tablets/Quart Iodine in PBDFW at pH 4.5, 7.0 and 9.5; 5°C and 25°C*

Virus	Time (Minutes) for 99.99% Inactivation at: pH 4.5	pH 7.0	pH 9.5
		5°C	
HAV	61	<1.0	<0.34**
Polio 1	>>60	<60	15
Echo 1	58	<11	<9.0
		25°C	
HAV	9.4	<0.4	<0.33
Polio 1	<12	4.0	<0.4
Echo 1	<15	<1.1	0.5

*Times for 99.99% inactivation estimated by linear regression analysis of $\log_{10}$ virus survival versus time; mean results of duplicate experiments for each condition.

**Less than (<) values are based on the detection limit for viruses in samples assayed; in cases where one replicate trial gave a measurable virus titer and the other replicate trial was indeterminate, the determinate value was averaged with the detection limit value.

FIGURE 2. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY FREE CHLORINE IN HDF WATER, 1 MG/L CL_2, pH 4.5, 5°C

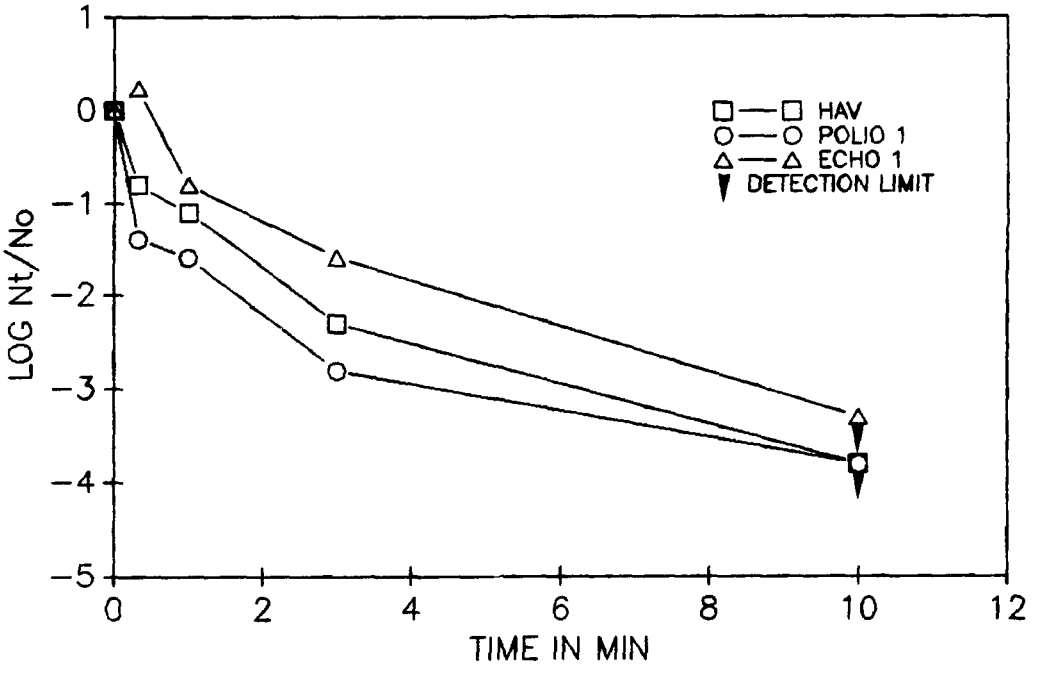

FIGURE 3. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY FREE CHLORINE IN HDF WATER, 1 MG/L Cl_2, pH 4.5, 25°C

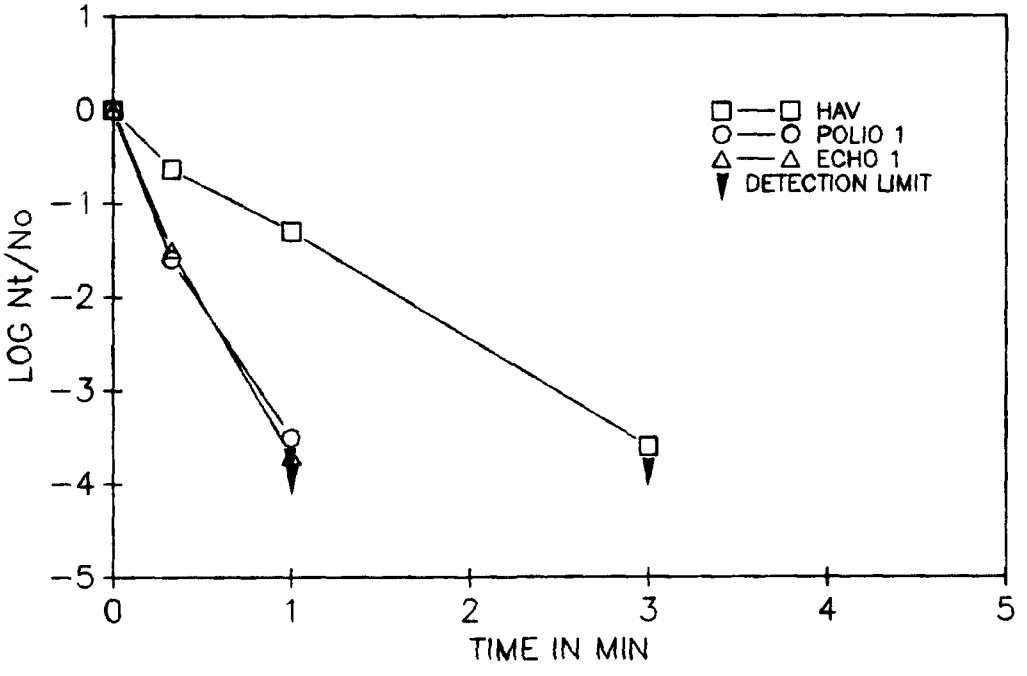

FIGURE 4. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY FREE CHLORINE IN HDF WATER, 1 MG/L CL_2, pH 7.0, 5°C

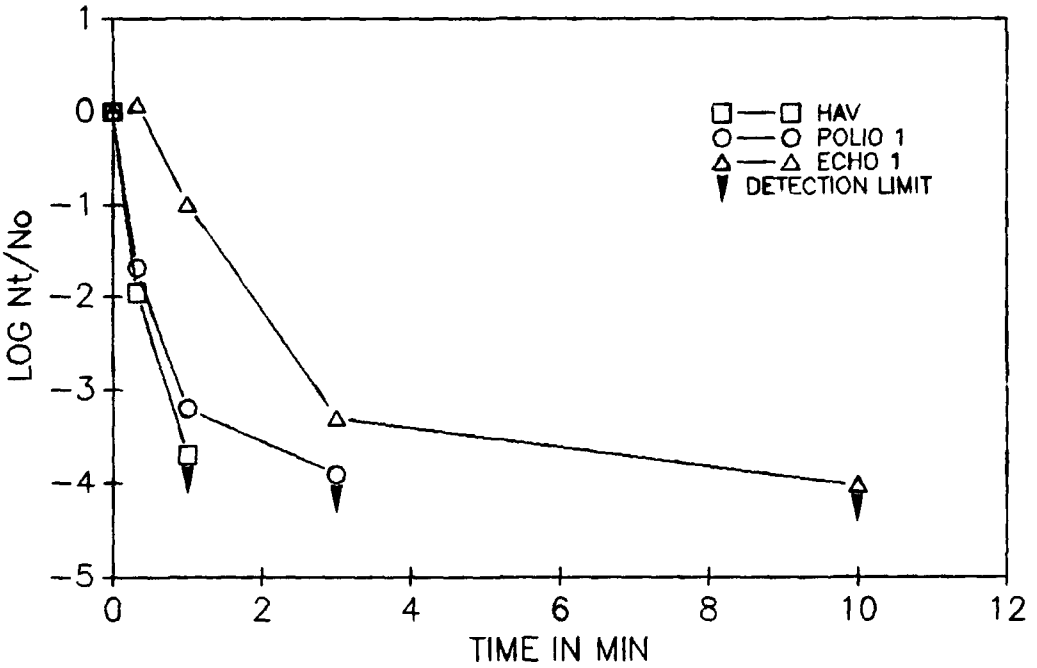

FIGURE 5. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY FREE CHLORINE IN HDF WATER, 1 MG/L CL_2, pH 7.0, 25°C

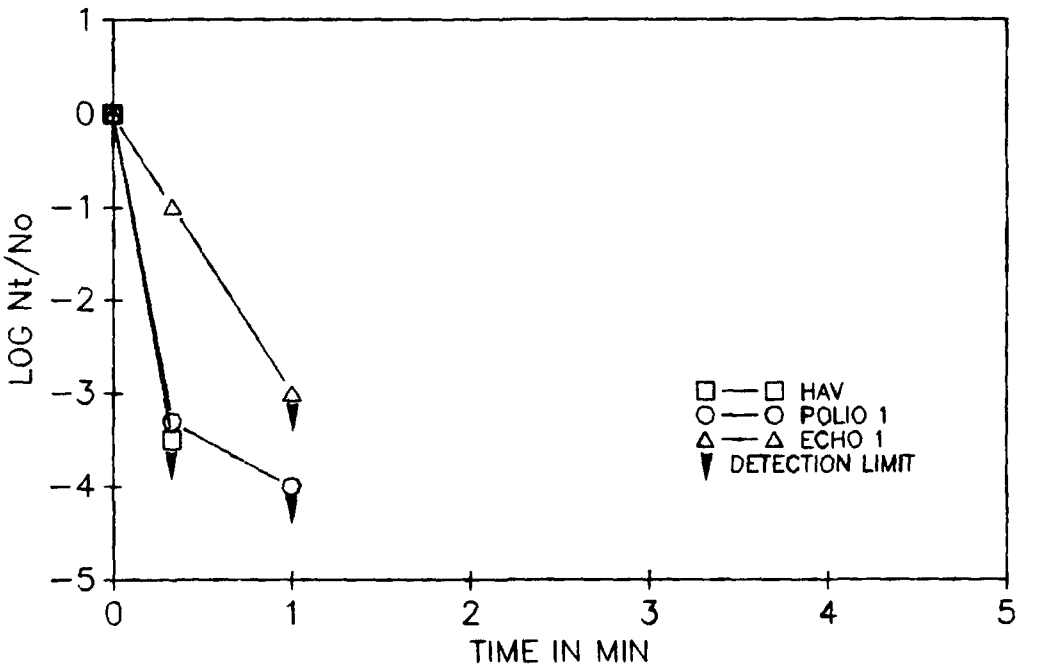

FIGURE 6. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY FREE CHLORINE IN HDF WATER, 1 MG/L Cl_2, pH 9.5, 5°C

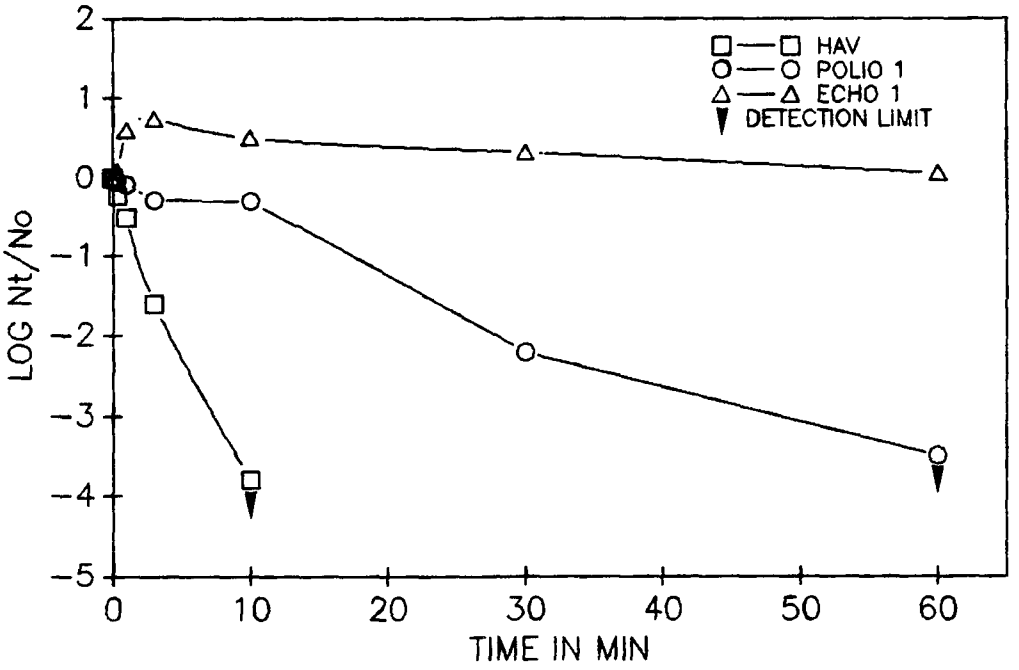

FIGURE 7. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY FREE CHLORINE IN HDF WATER, 1 MG/L CL_2, pH 9.5, 25°C

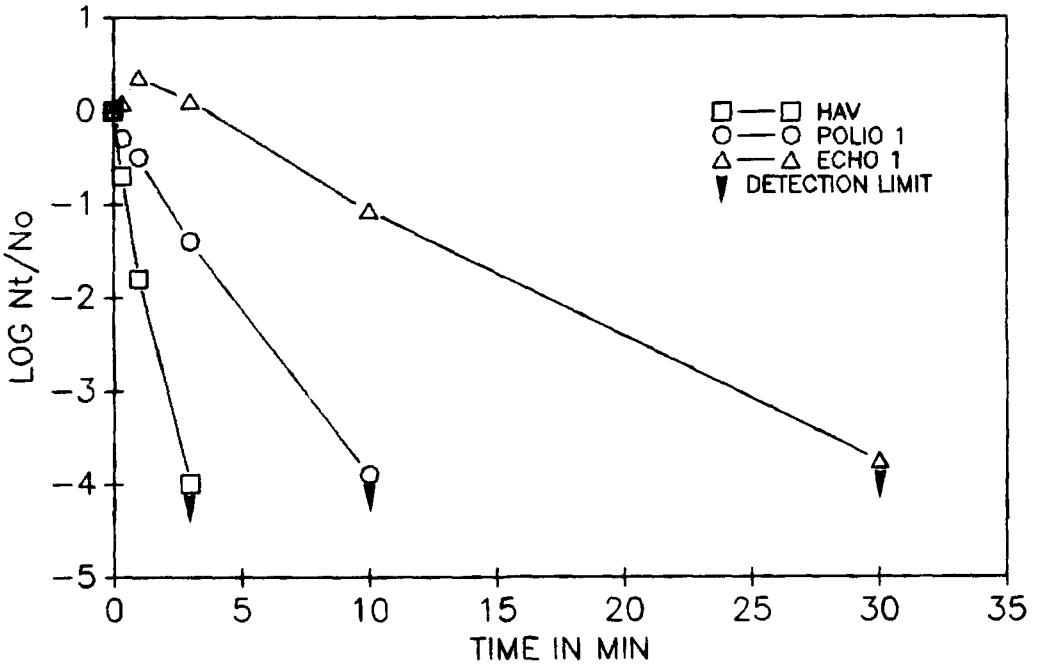

FIGURE 8. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY FREE CHLORINE IN HDF WATER, 5 MG/L CL_2, pH 4.5, 5°C

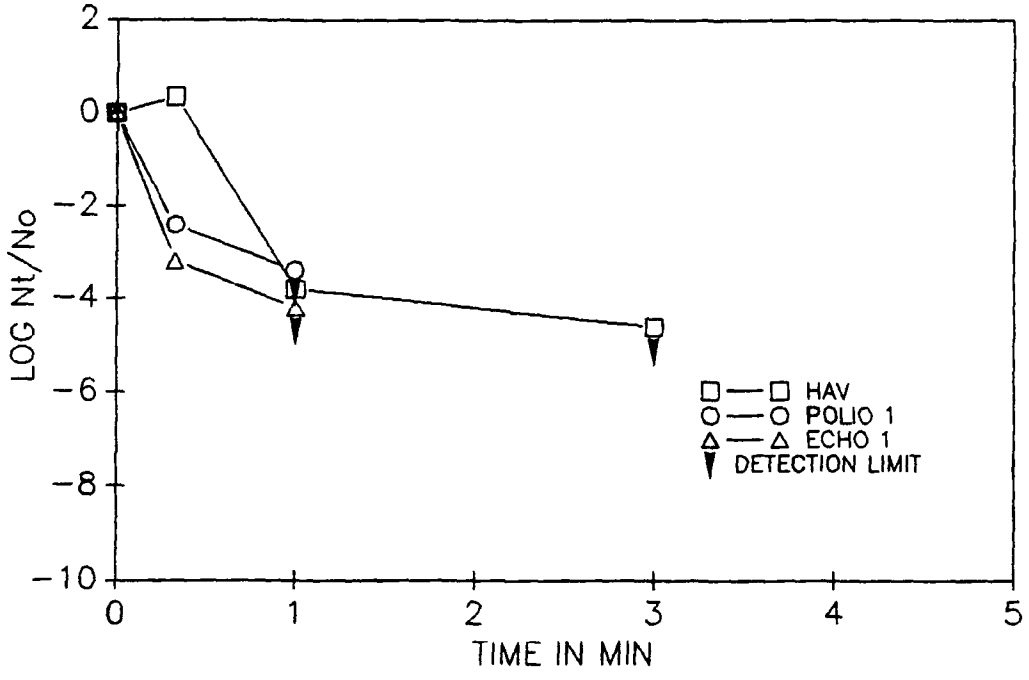

FIGURE 9. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY FREE CHLORINE IN HDF WATER, 5 MG/L Cl_2, pH 4.5, 25°C

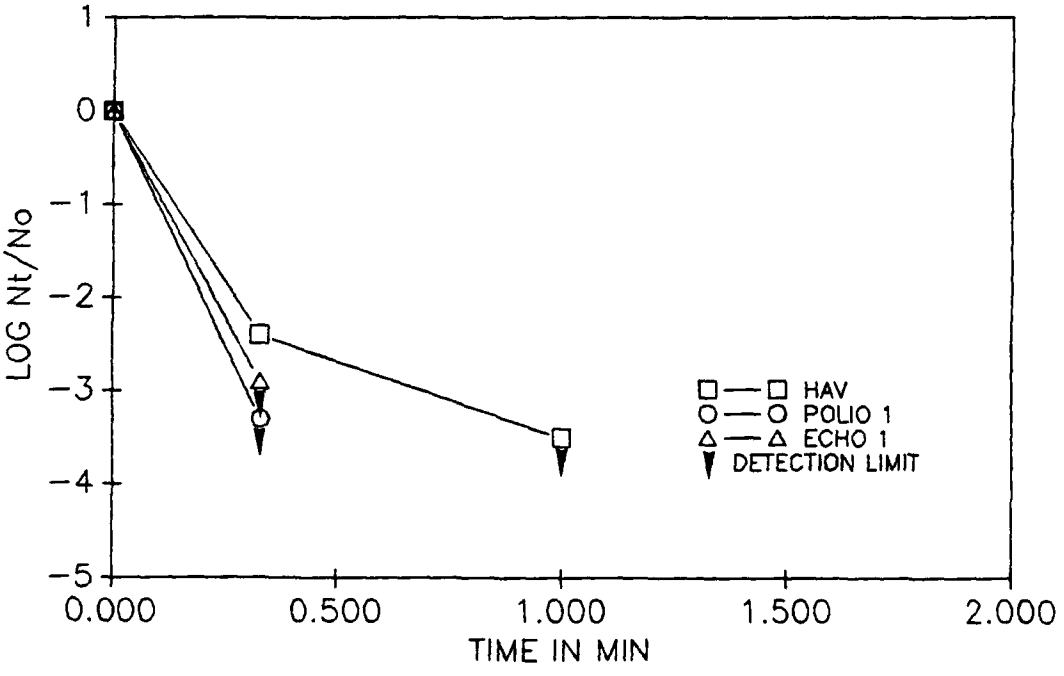

FIGURE 10. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY FREE CHLORINE IN HDF WATER, 5 MG/L CL_2, pH 7.0, 5°C

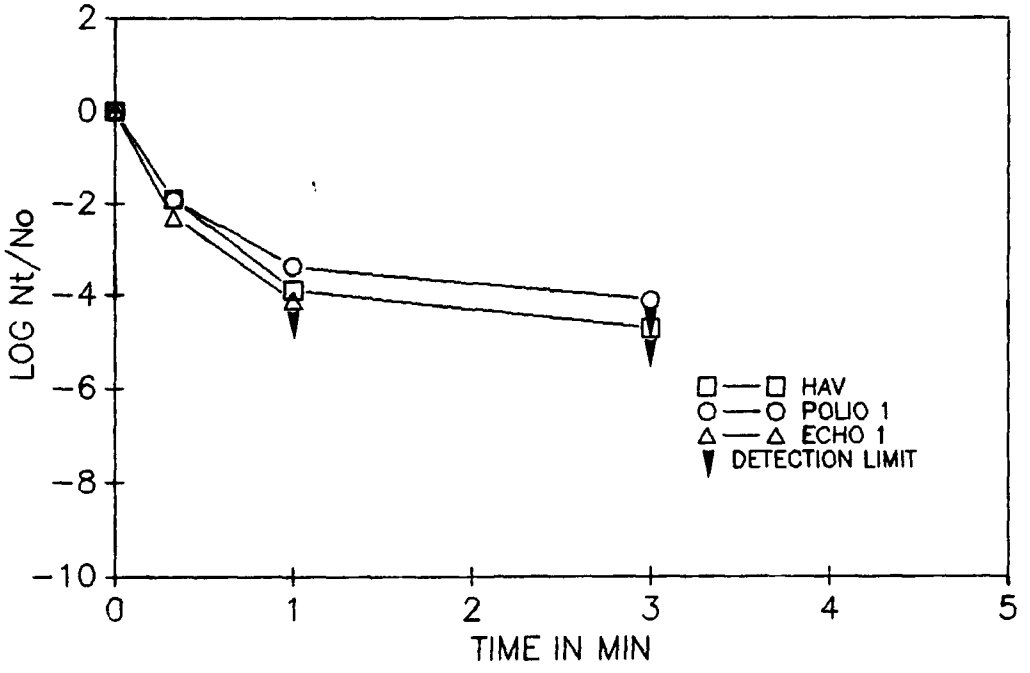

FIGURE 11. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY FREE CHLORINE IN HDF WATER, 5 MG/L CL_2, pH 7.0, 25°C

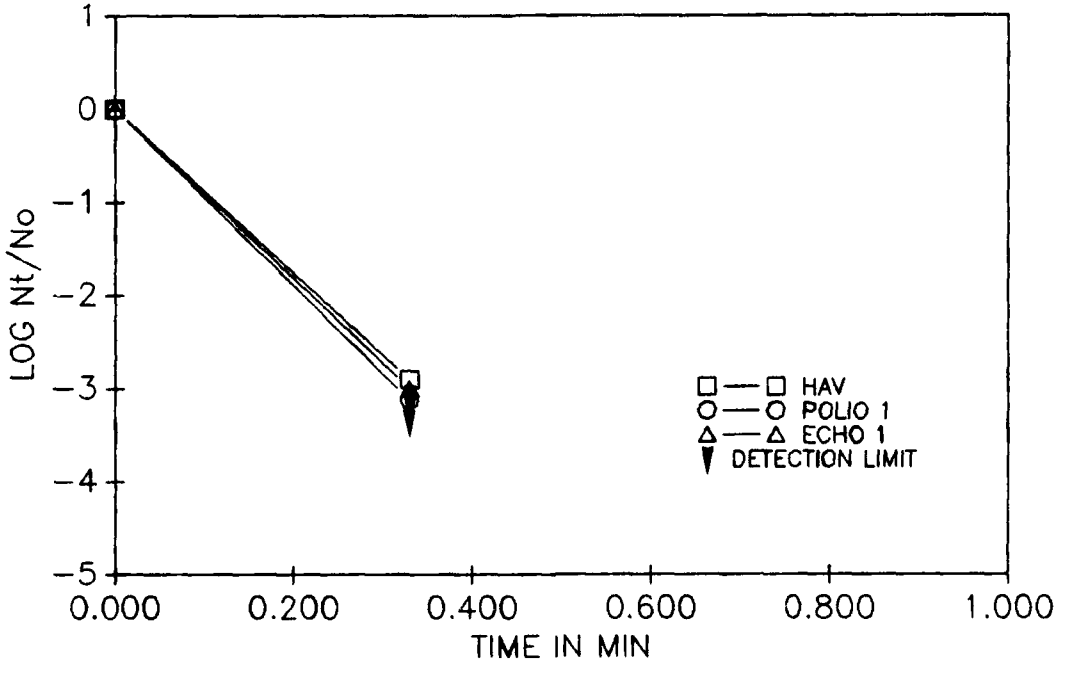

FIGURE 12. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY FREE CHLORINE IN HDF WATER, 5 MG/L CL_2, pH 9.5, 5°C

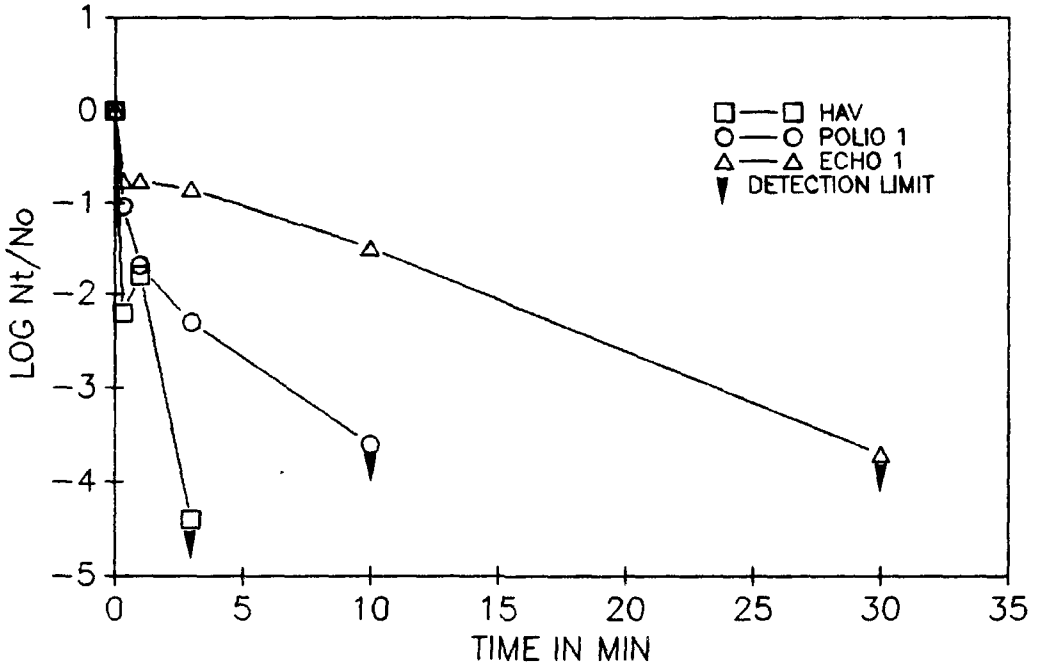

FIGURE 13. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY FREE CHLORINE IN HDF WATER, 5 MG/L CL_2, pH 9.5, 25°C

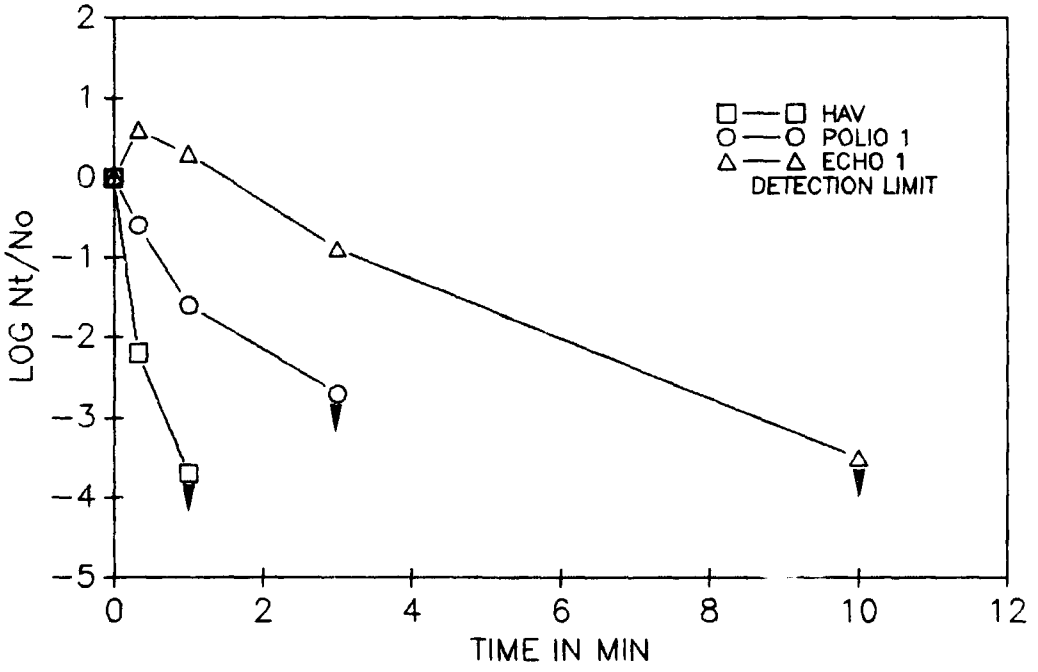

FIGURE 14. INACTIVATION OF HAV STRAINS HM−175 AND MD−1 AND ECHO 1 BY 1 MG/L FREE CHLORINE, pH 9.5 AND 5°C

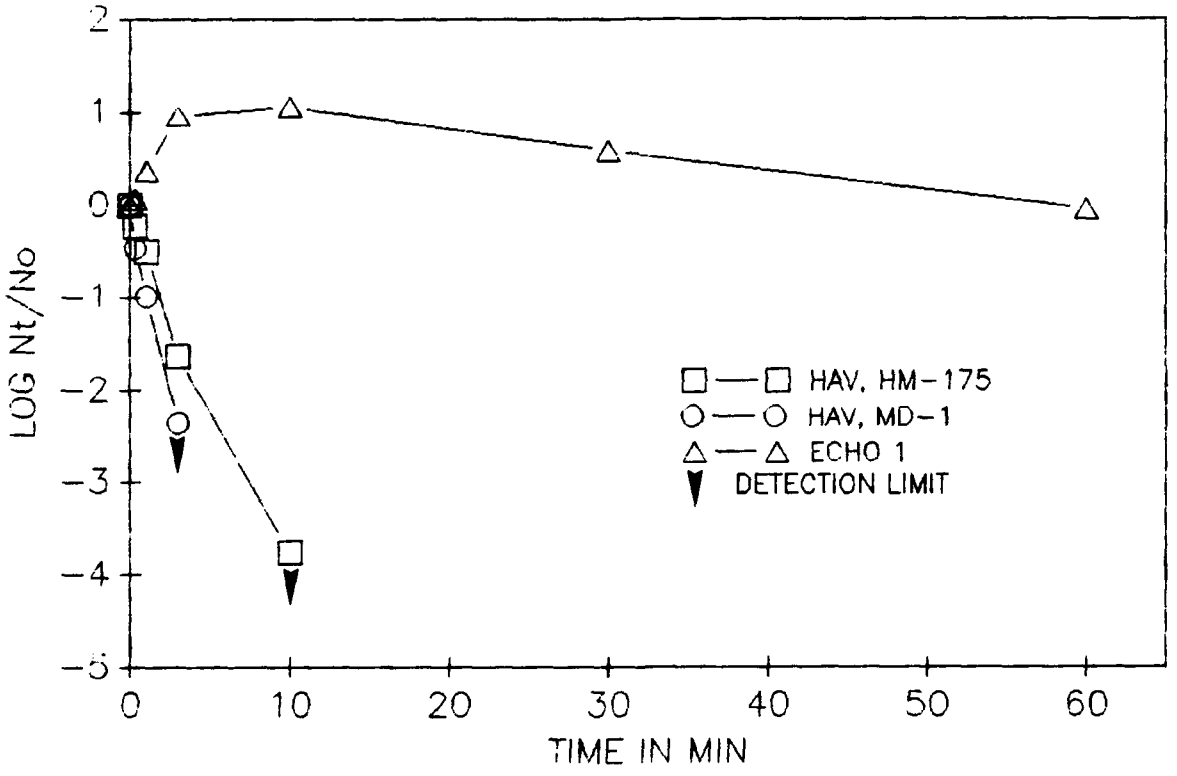

FIGURE 15. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY IODINE IN HDF WATER, 1 TABLET/QUART, pH 4.5, 5°C

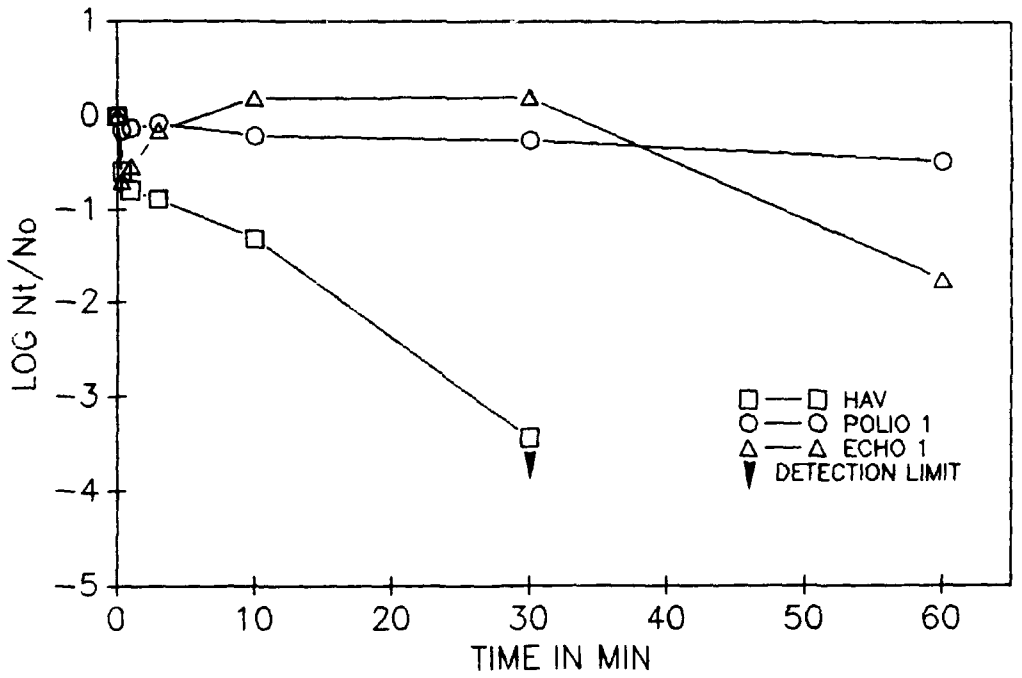

FIGURE 16. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY IODINE IN HDF WATER, 1 TABLET/QUART, pH 4.5, 25°C

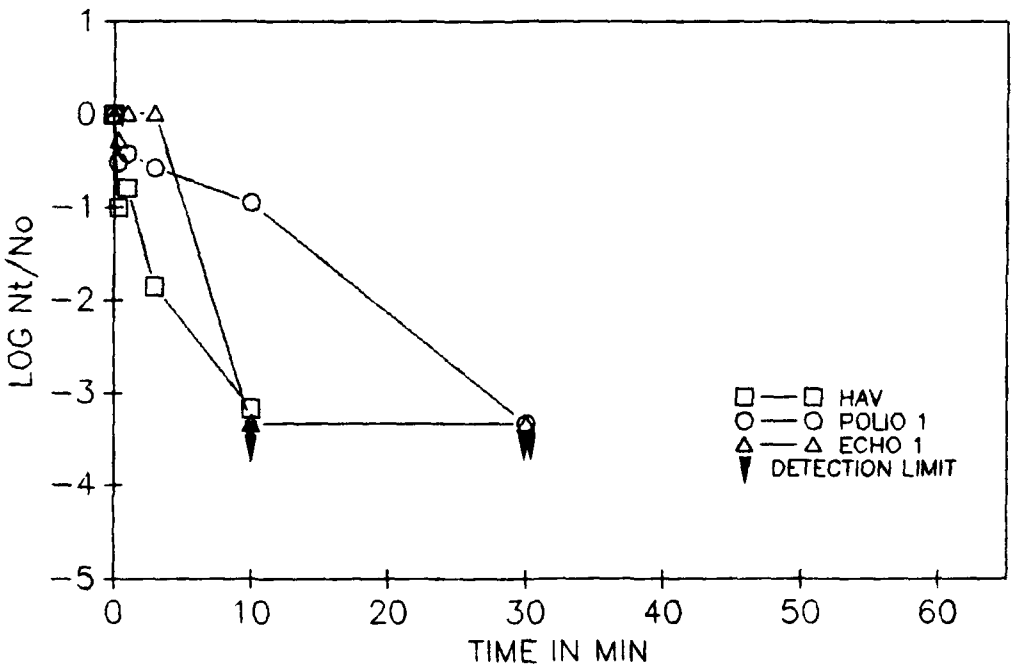

FIGURE 17. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY IODINE IN HDF WATER, 1 TABLET/QUART, pH 7.0, 5°C

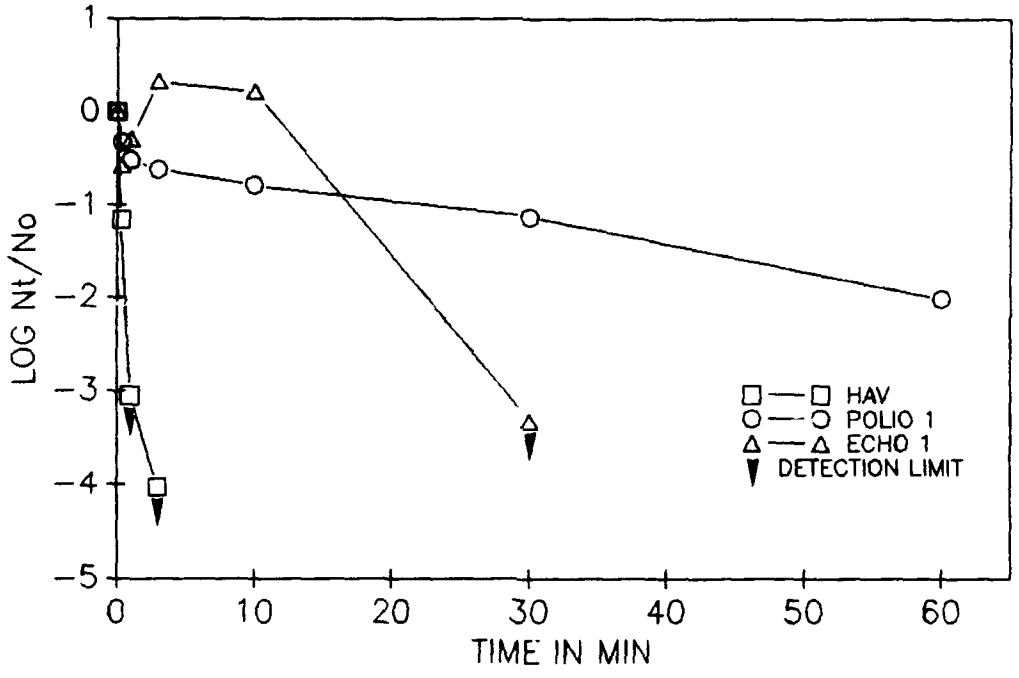

FIGURE 18. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY IODINE IN HDF WATER, 1 TABLET/QUART, pH 7.0, 25°C

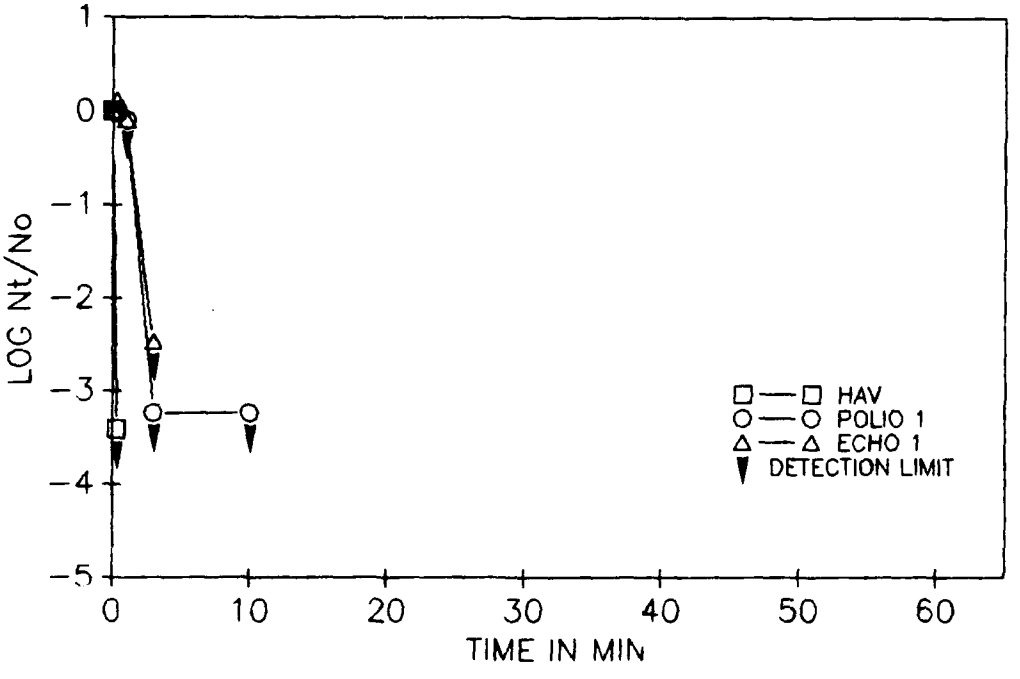

FIGURE 19. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY IODINE IN HDF WATER, 1 TABLET/QUART, pH 9.5, 5°C

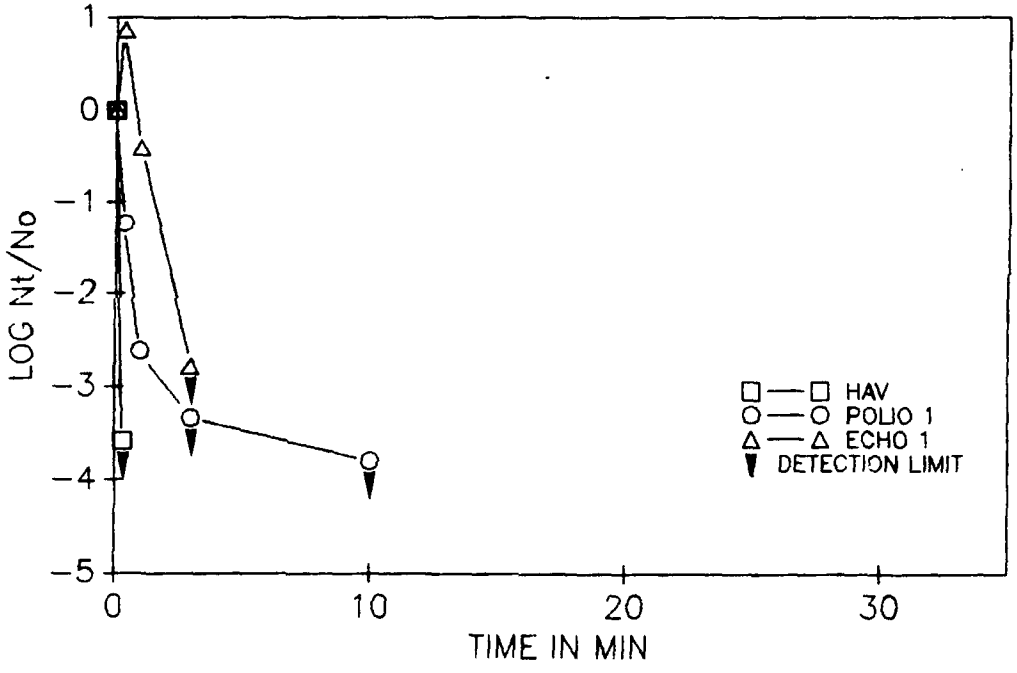

FIGURE 20. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY IODINE IN HDF WATER, 1 TABLET/QUART, pH 9.5, 25°C

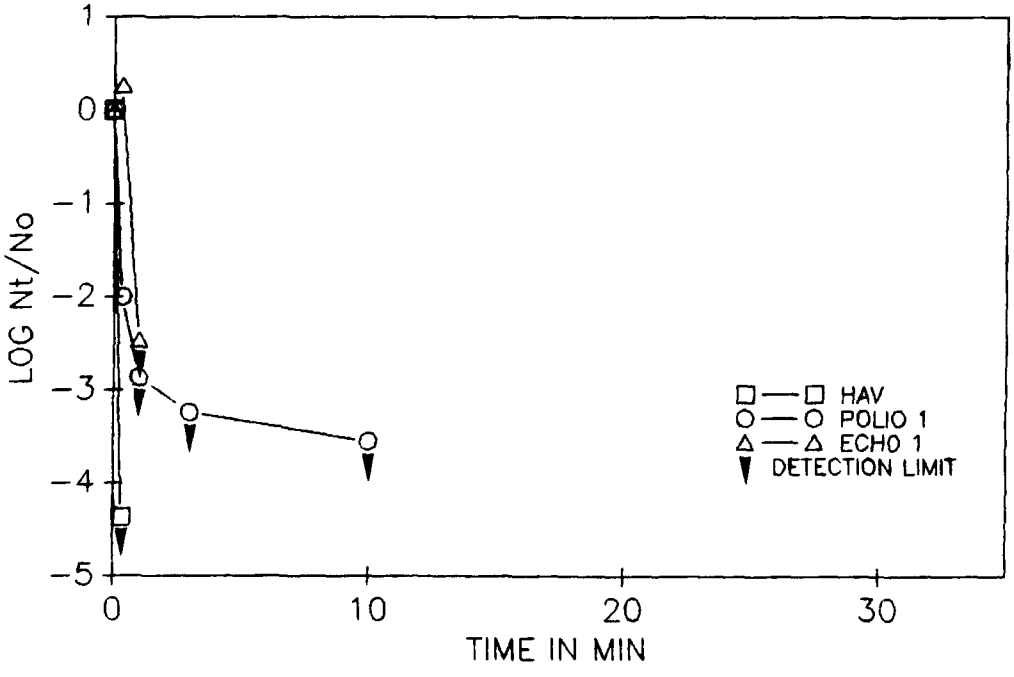

FIGURE 21. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY IODINE IN HDF WATER, 2 TABLETS/QUART, pH 4.5, 5°C

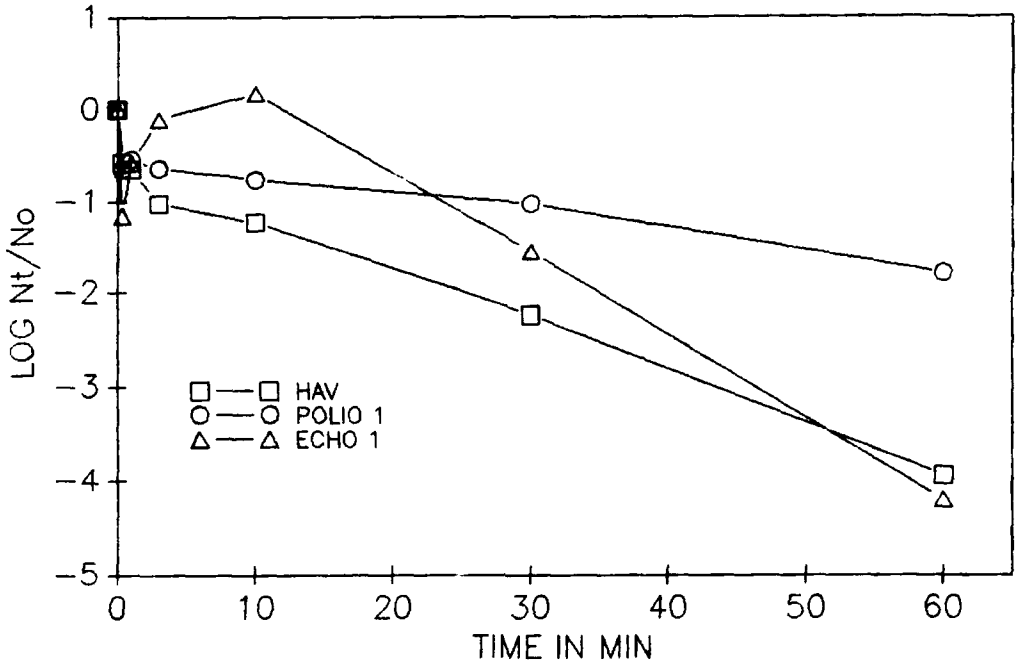

FIGURE 22. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY IODINE IN HDF WATER, 2 TABLETS/QUART, pH 4.5, 25°C

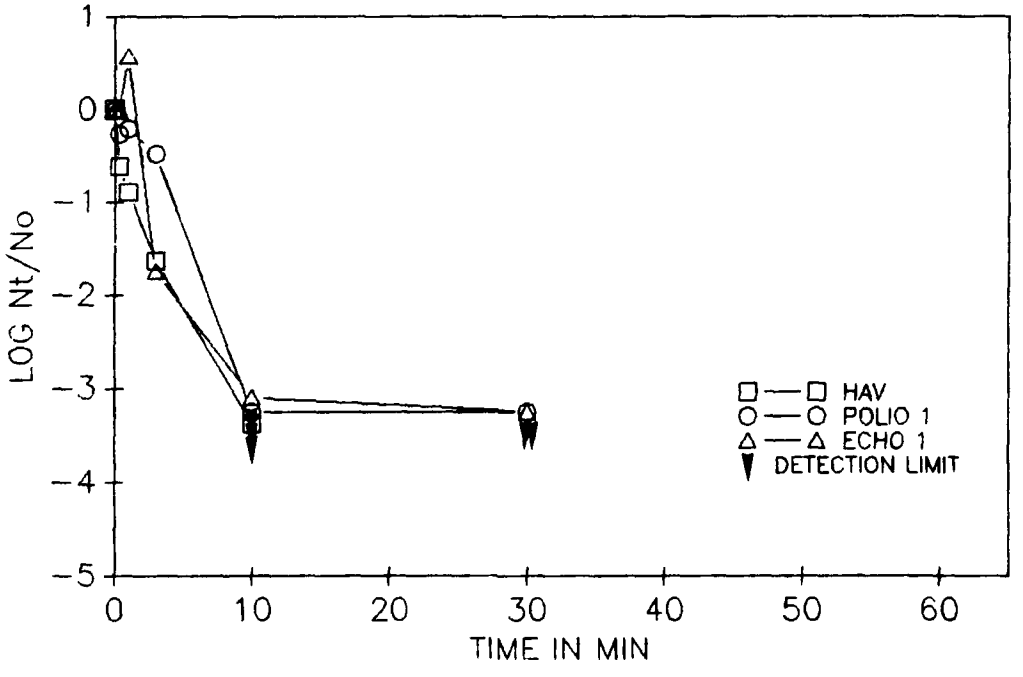

FIGURE 23. INACTIVATION OF HAV, POLIO 1, AND ECHO 1 BY IODINE IN HDF WATER, 2 TABLETS/QUART, pH 7.0, 5°C

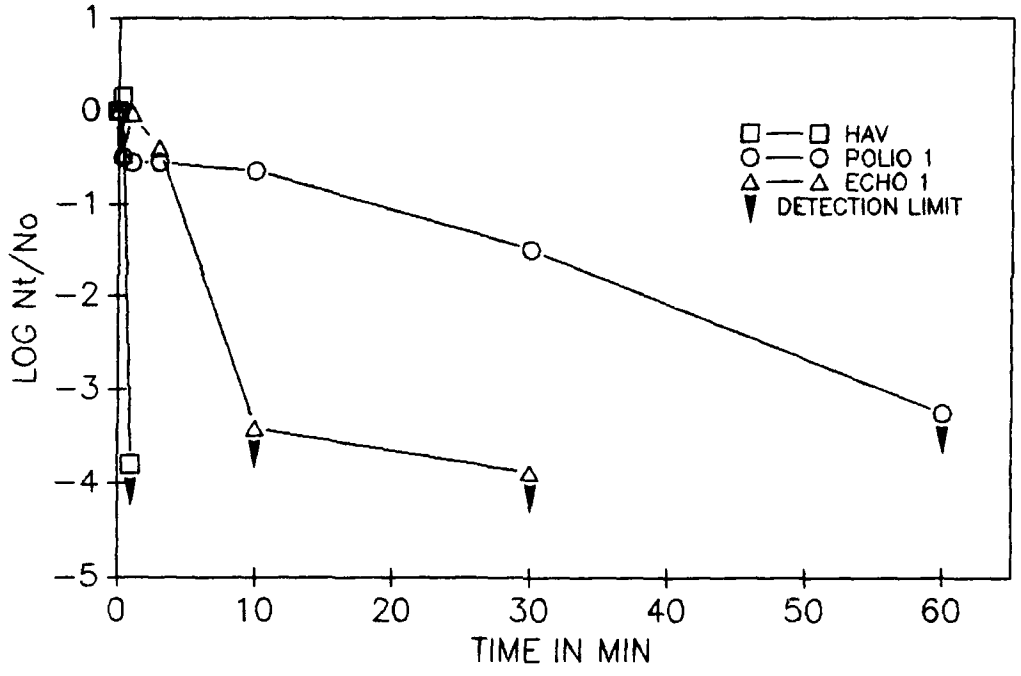

FIGURE 24. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY IODINE IN HDF WATER, 2 TABLETS/QUART, pH 7.0, 25°C

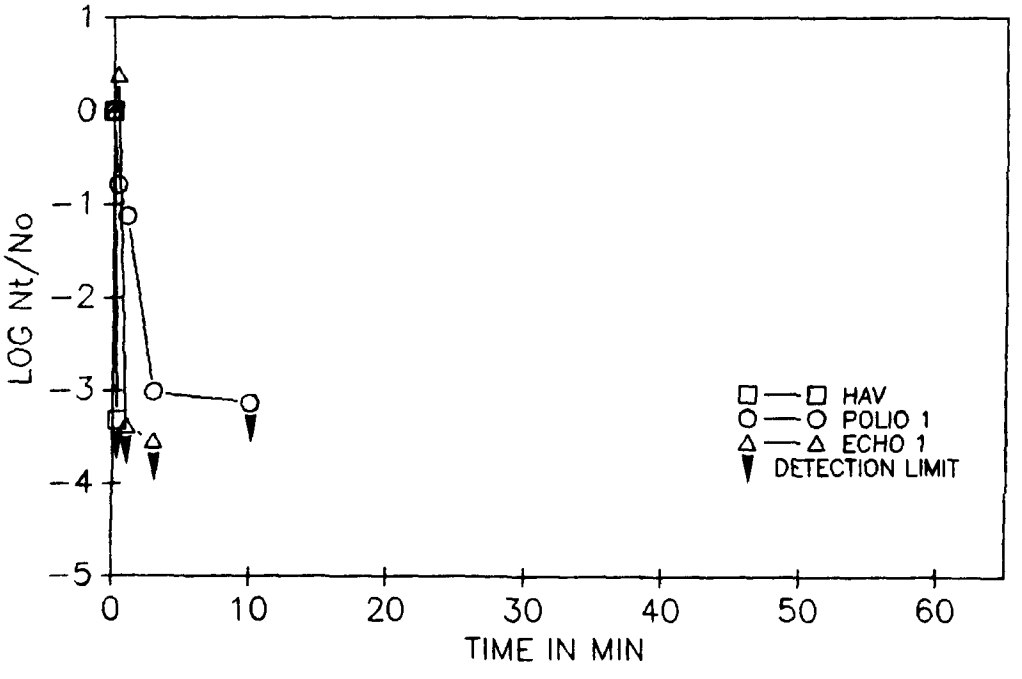

FIGURE 25. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY IODINE IN HDF WATER, 2 TABLETS/QUART, pH 9.5, 5°C

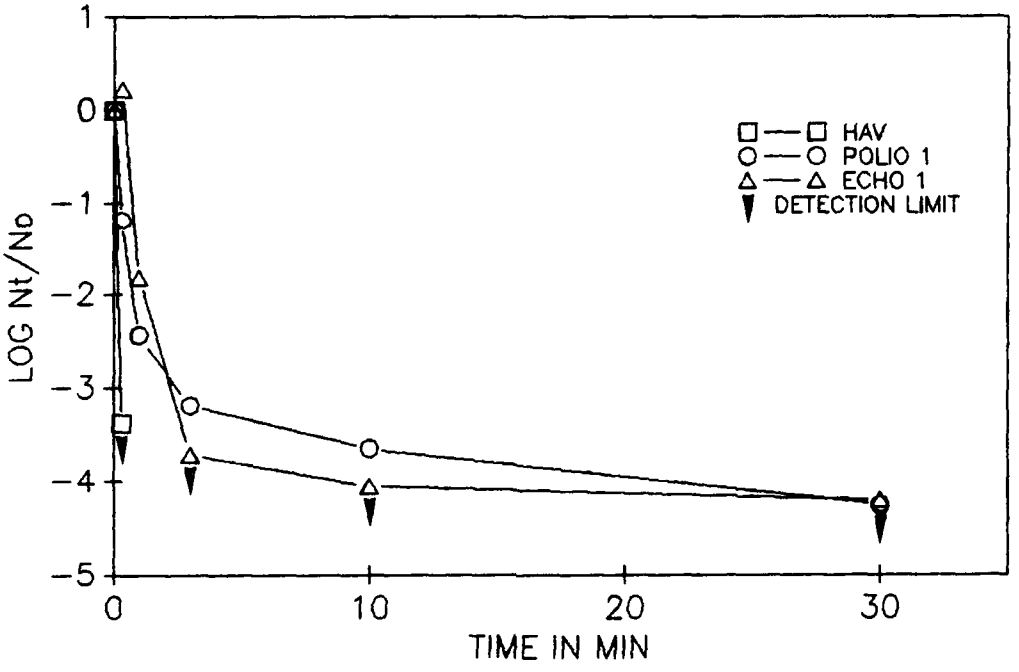

FIGURE 26. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY IODINE IN HDF WATER, 2 TABLETS/QUART, pH 9.5, 25°C

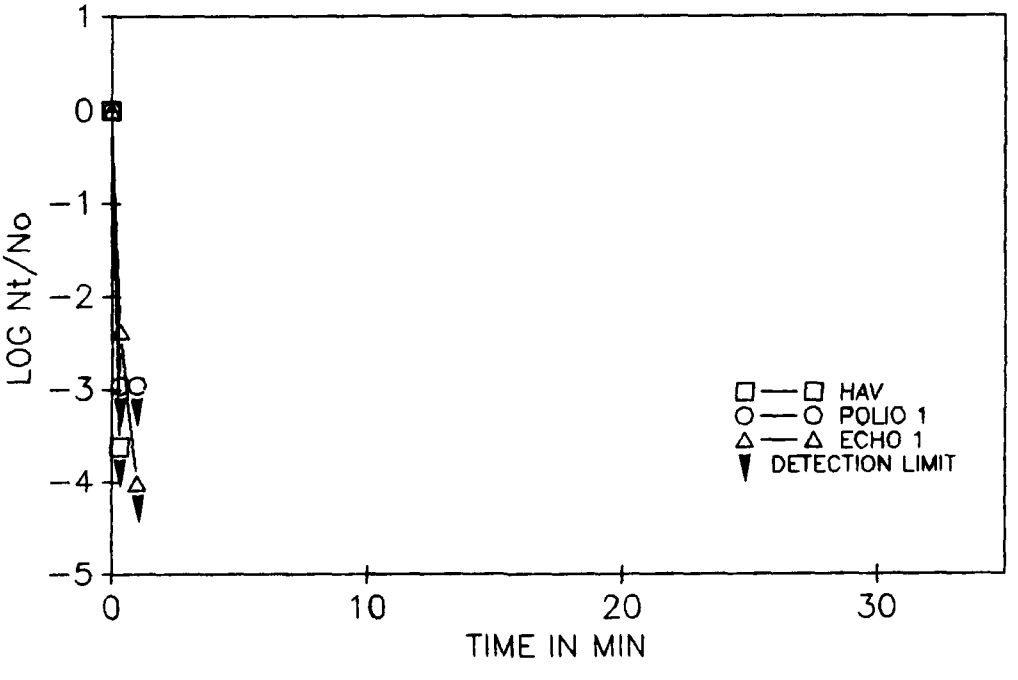

FIGURE 27. STABILITY OF 1MG/L FREE CHLORINE IN PBHDFW, pH 4.5, 7.0 AND 9.5 AND 5°C

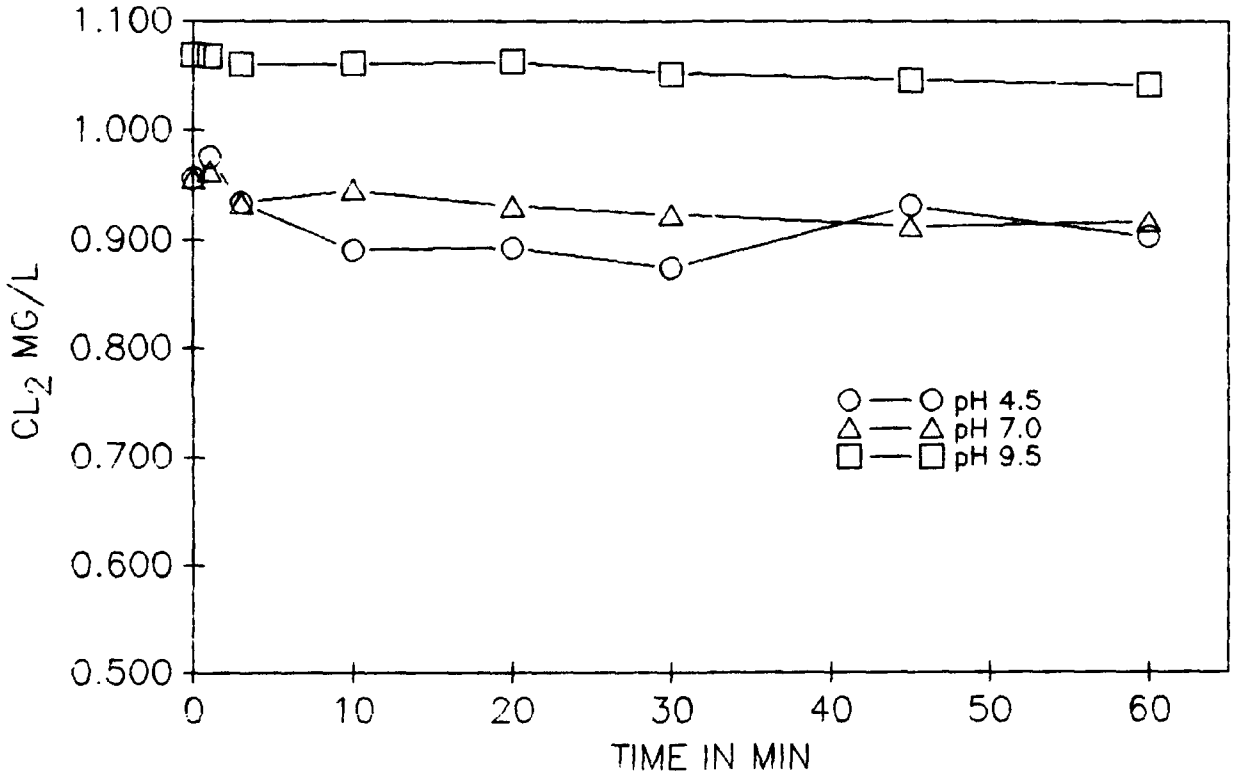

FIGURE 28. STABILITY OF 1 MG/L FREE CHLORINE IN PBHDFW pH 4.5, 7.0 AND 9.5 AND 25 °C

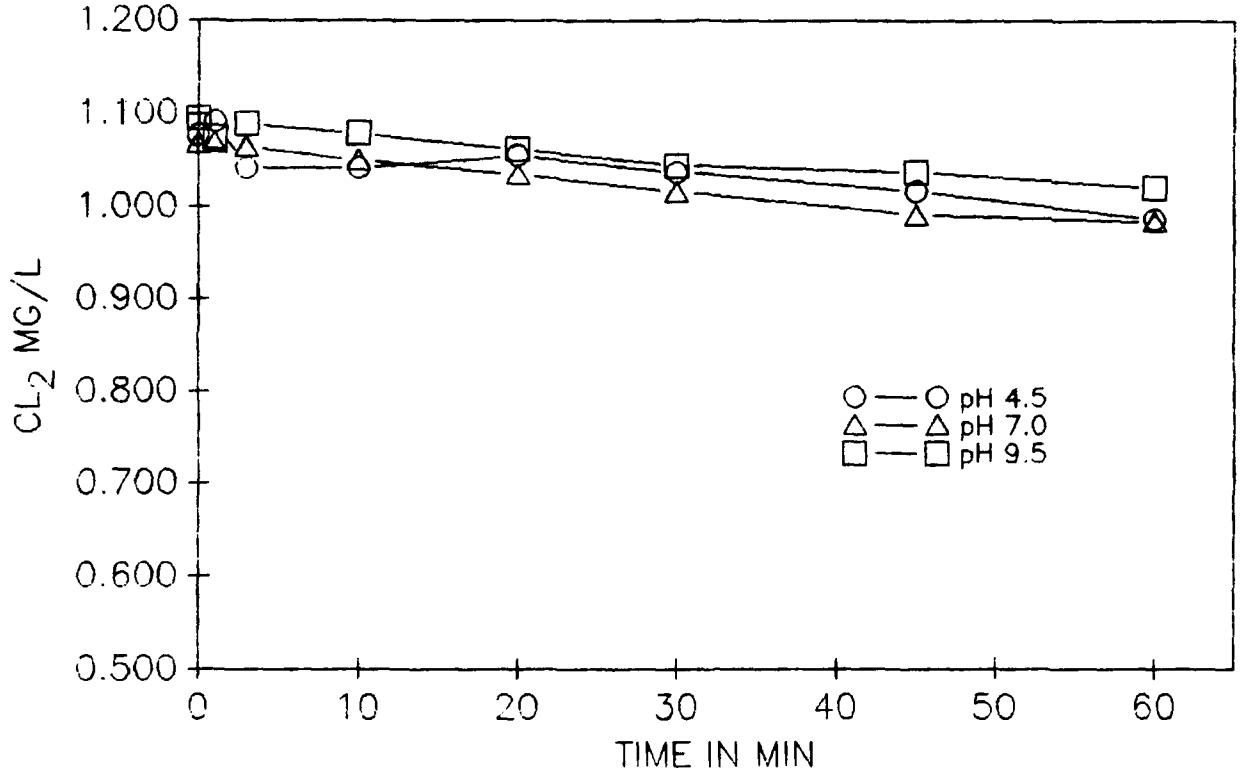

FIGURE 29. STABILITY OF 5 MG/L FREE CHLORINE IN PBHDFW, pH 9.5 AND 25 °C

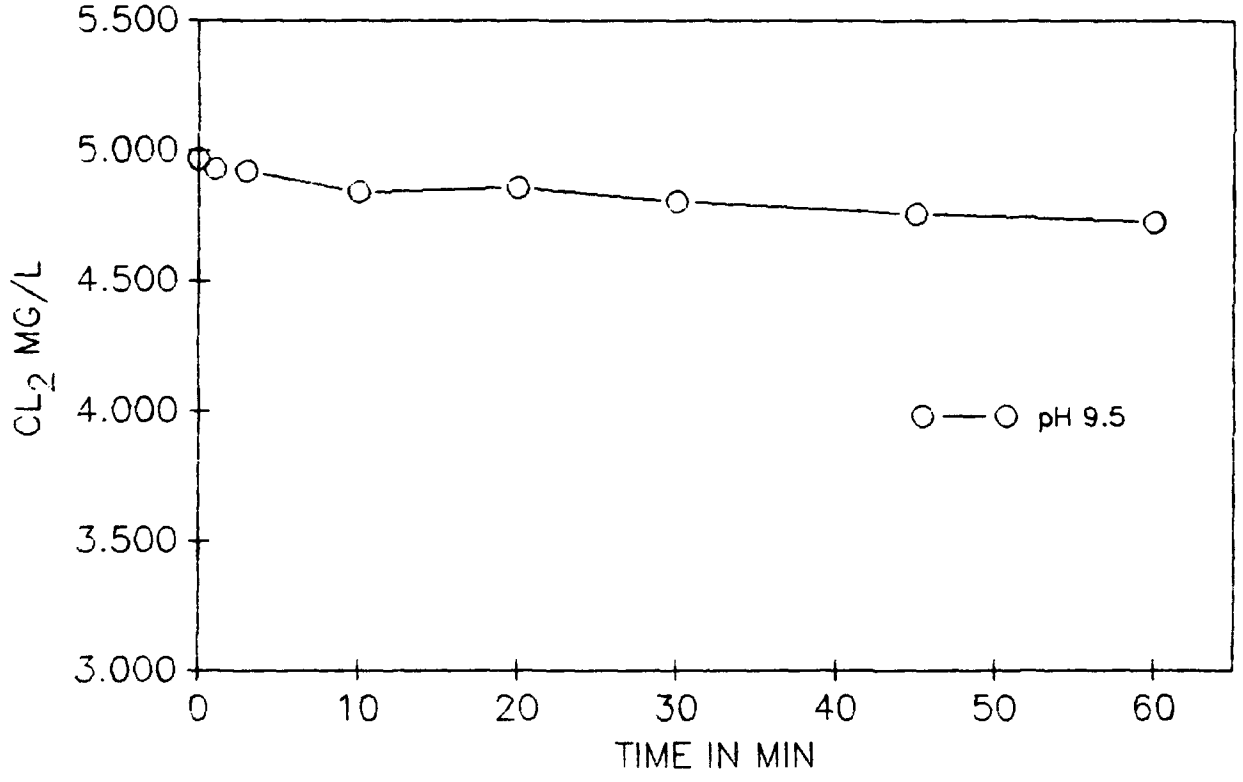

FIGURE 30. STABILITY OF 1 TABLET/QUART IODINE IN PBHDFW, pH 4.5, 7.0 AND 9.5 AND 5°C

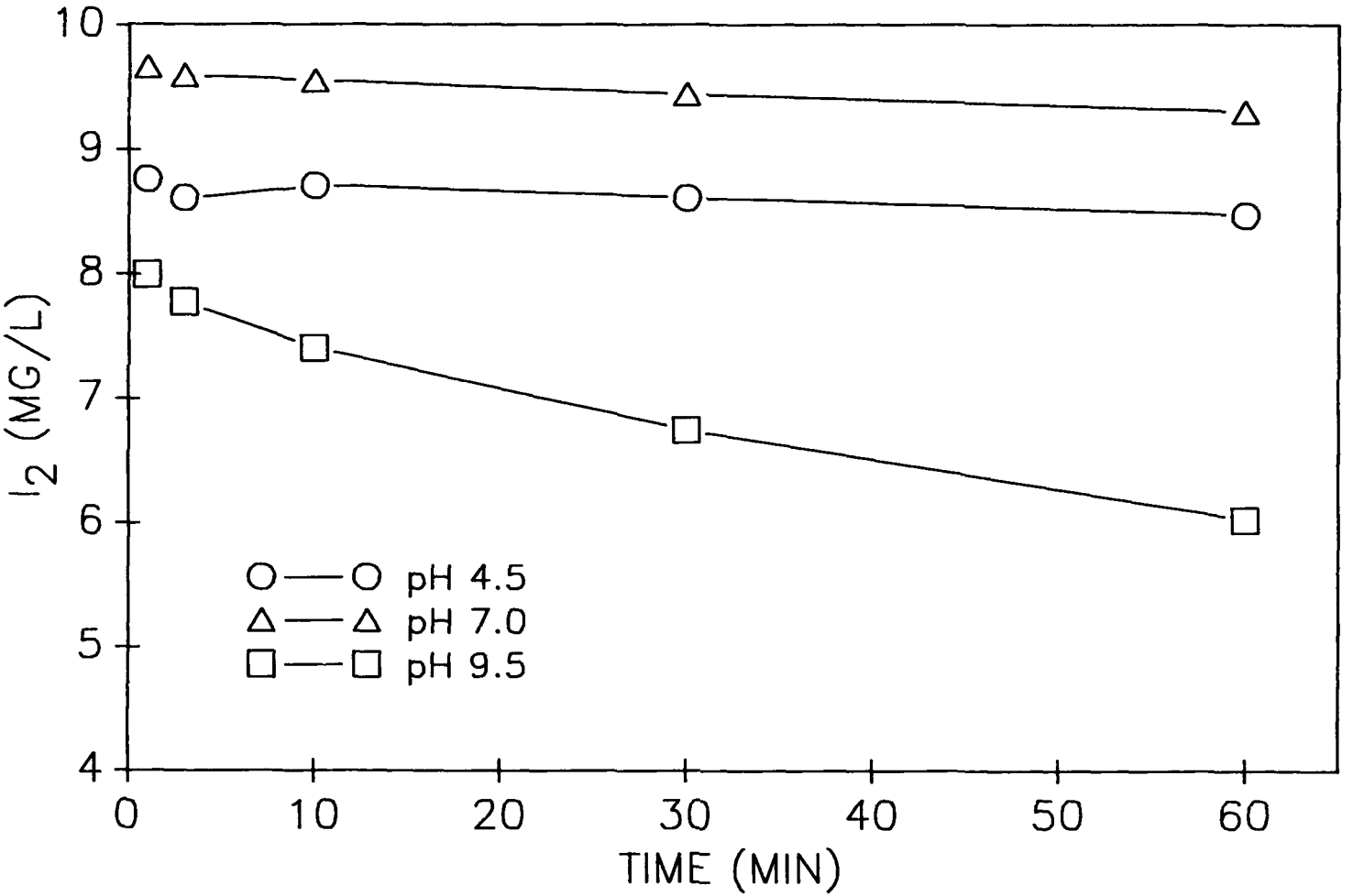

FIGURE 31. STABILITY OF 1 TABLET/QUART IODINE IN PBHDFW, pH 4.5, 7.0 AND 9.5 AND 25°C

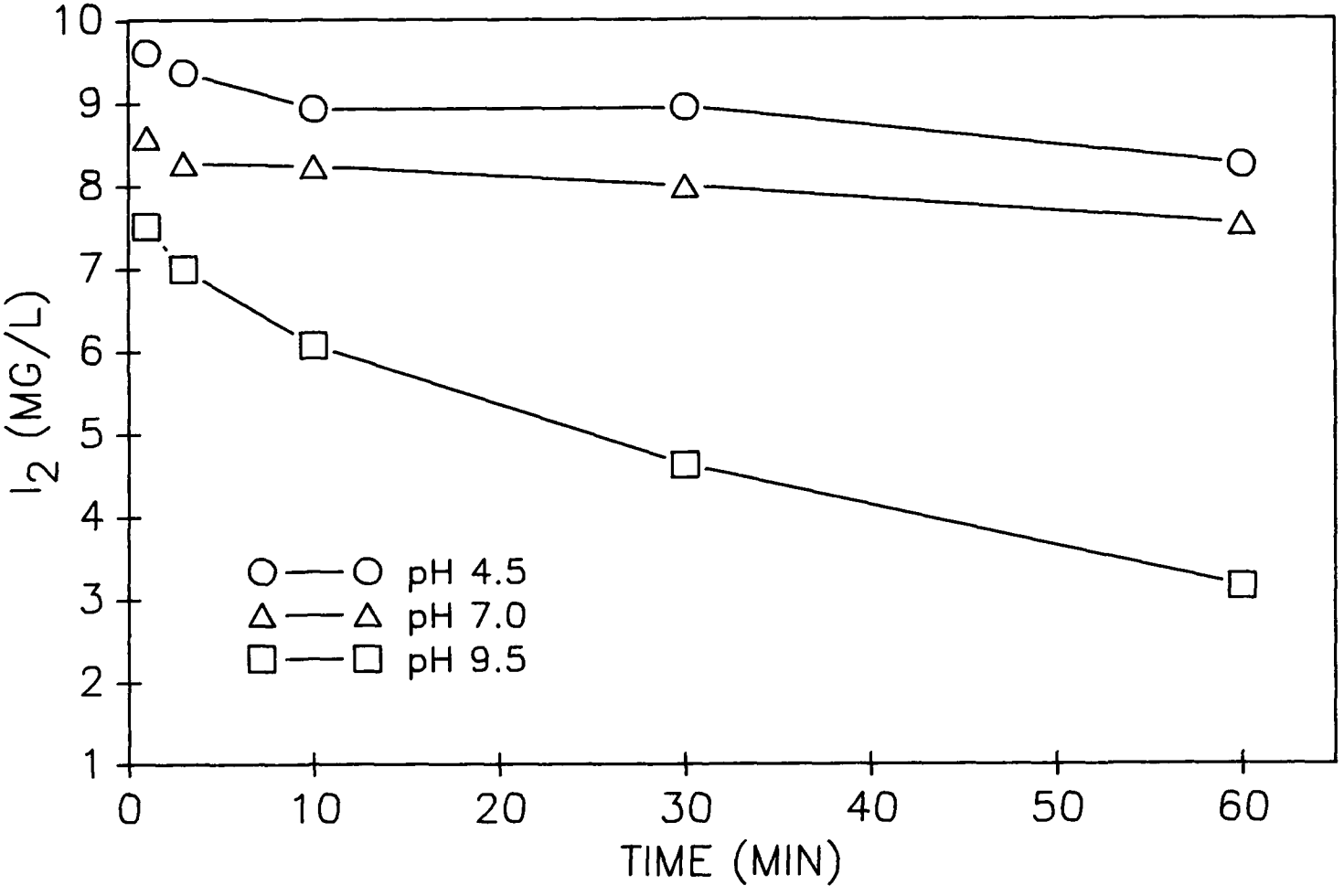

Table A1. Inactivation of HAV, Polio 1 and Echo 1 by 1 mg/l Free Chlorine at pH 4.5 and 5 C.

AVG CL2 CONCENTRATION 0'= 1.08 mg/l
60'= 0.04 mg/l

VIRUS = HAV

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	2.80E+03	5.00E-02	9.33E+03	2.63E-01	1.56E-01	-0.81
TS-1'	4.67E+03	8.34E-02	2.80E+03	7.89E-02	8.11E-02	-1.09
TS-3'	4.67E+02	8.34E-03	1.02E+02	2.87E-03	5.61E-03	-2.25
TS-10'	<6.67E+00	1.19E-04	<6.67E+00	1.88E-04	1.53E-04	>-3.81
TS-30'						
TS-60'						
AVG VC	5.60E+04		3.55E+04			
VC-0'	8.40E+04		4.11E+04			
VC-60'	2.80E+04		2.99E+04			

VIRUS= POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	1.68E+03	3.63E-02	1.12E+03	5.23E-02	4.43E-02	-1.35
TS-1'	8.40E+02	1.82E-02	6.53E+02	3.05E-02	2.43E-02	-1.61
TS-3'	<6.67E+00	1.44E-04	6.53E+01	3.05E-03	1.60E-03	-2.80
TS-10'			<6.67E+00	3.12E-04	1.56E-04	>-3.81
TS-30'						
TS-60'						
AVG VC	4.63E+04		2.14E+04			
VC-0'	3.74E+04		2.60E+04			
VC-60'	5.51E+04		1.68E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	1.73E+04	5.88E-01	2.24E+04	2.82E+00	1.71E+00	0.23
TS-1'	2.71E+03	9.22E-02	1.77E+03	2.23E-01	1.58E-01	-0.80
TS-3'	8.40E+01	2.86E-03	3.73E+02	4.70E-02	2.49E-02	-1.60
TS-10'	<6.67E+00	2.27E-04	<6.67E+00	8.41E-04	5.34E-04	>-3.27
TS-30'						
TS-60'						
AVG VC	2.94E+04		7.94E+03			
VC-0'	1.77E+04		7.47E+03			
VC-60'	4.11E+04		8.40E+03			

Table A2. Inactivation of HAV, Polio 1 and Echo 1 by 1 mg/l Free Chlorine at pH 7.0 and 5 C.

AVG CL2 CONCENTRATION 0'= 0.98 mg/l
60'= 0.32 mg/l

VIRUS = HAV

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	1.31E+02	5.10E-03	9.33E+02	1.67E-02	1.09E-02	-1.96
TS-1'	<6.67E+00	2.60E-04	<6.67E+00	1.19E-04	1.89E-04	>-3.72
TS-3'						
TS-10'						
TS-30'						
TS-60'						
AVG VC	2.57E+04		5.60E+04			
VC-0'	2.71E+04		6.53E+04			
VC-60'	2.43E+04		4.67E+04			

VIRUS= POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	4.76E+01	6.54E-04	1.77E+03	4.27E-02	2.17E-02	-1.66
TS-1'	2.80E+01	3.85E-04	3.72E+01	8.96E-04	6.41E-04	-3.19
TS-3'	<6.67E+00	9.16E-05	<6.67E+00	1.61E-04	1.26E-04	>-3.90
TS-10'						
TS-30'						
TS-60'						
AVG VC	7.28E+04		4.15E+04			
VC-0'	9.33E+04		4.57E+04			
VC-60'	5.23E+04		3.73E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	2.24E+04	6.32E-01	7.19E+04	1.79E+00	1.21E+00	0.08
TS-1'	5.60E+02	1.58E-02	7.46E+03	1.86E-01	1.01E-01	-1.00
TS-3'	2.80E+01	7.90E-04	<6.67E+00	1.66E-04	4.78E-04	-3.32
TS-10'	<6.67E+00	1.88E-04			9.41E-05	>-4.03
TS-30'						
TS-60'						
AVG VC	3.55E+04		4.02E+04			
VC-0'	3.64E+04		2.80E+04			
VC-60'	3.45E+04		5.23E+04			

Table A3. Inactivation of HAV, Polio 1 and Echo 1 by 1 mg/l Free Chlorine at pH 9.5 and 5 C.

AVG CL2 CONCENTRATION 0'= 1.1 mg/l
60'= 0.54 mg/l

VIRUS = HAV

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	1.21E+04	2.49E-01	2.89E+04	9.25E-01	5.87E-01	-0.23
TS-1'	1.87E+03	3.85E-02	1.87E+04	5.98E-01	3.18E-01	-0.50
TS-3'	3.73E+02	7.68E-03	1.21E+03	3.87E-02	2.32E-02	-1.63
TS-10'	<6.67E+00	1.37E-04	<6.67E+00	2.13E-04	1.75E-04	>-3.76
TS-30'						
TS-60'						
AVG VC	4.86E+04		3.13E+04			
VC-0'	3.73E+04		3.64E+04			
VC-60'	5.98E+04		2.61E+04			

VIRUS= POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	1.68E+04	8.00E-01	2.33E+04	9.79E-01	8.89E-01	-0.05
TS-1'	1.96E+04	9.33E-01	1.59E+04	6.68E-01	8.01E-01	-0.10
TS-3'	1.03E+04	4.90E-01	1.31E+04	5.50E-01	5.20E-01	-0.28
TS-10'	1.59E+04	7.57E-01	5.64E+03	2.37E-01	4.97E-01	-0.30
TS-30'	3.73E+01	1.78E-03	2.61E+02	1.10E-02	6.37E-03	-2.20
TS-60'	< 6.67E+00	3.18E-04	<6.67E+00	2.80E-04	2.99E-04	>-3.52
AVG VC	2.10E+04		2.38E+04			
VC-0'	2.05E+04		1.96E+04			
VC-60'	2.15E+04		2.80E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	1.12E+05	1.71E+00	1.87E+04	7.15E-01	1.21E+00	0.08
TS-1'	1.12E+05	1.71E+00	1.40E+05	5.35E+00	3.53E+00	0.55
TS-3'	1.87E+05	2.86E+00	2.15E+05	8.22E+00	5.54E+00	0.74
TS-10'	0.00E+00	0.00E+00	1.60E+05	6.12E+00	3.06E+00	0.49
TS-30'	1.86E+04	2.85E-01	1.03E+05	3.94E+00	2.11E+00	0.32
TS-60'	1.12E+04	1.71E-01	5.60E+04	2.14E+00	1.16E+00	0.06
AVG VC	6.54E+04		2.62E+04			
VC-0'	7.47E+04		9.33E+03			
VC-60'	5.60E+04		4.30E+04			

Table A4. Inactivation of HAV, Polio 1 and Echo 1 by 1 mg/l Free Chlorine at pH 4.5 and 25 C.

AVG CL2 CONCENTRATION 0'= 0.98 mg/l
60'= 0.12 mg/l

VIRUS = HAV

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	4.90E+03	2.58E-01	8.80E+03	1.98E-01	2.28E-01	-0.64
TS-1'	1.73E+03	9.11E-02	3.70E+02	8.34E-03	4.97E-02	-1.30
TS-3'	<6.67E+00	3.51E-04	<6.67E+00	1.50E-04	2.51E-04	>-3.60
TS-10'						
TS-30'						
TS-60'						
AVG VC	1.90E+04		4.44E+04			
VC-0'	3.80E+04		5.27E+04			
VC-60'	0.00E+00		3.60E+04			

VIRUS= POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	4.20E+02	2.80E-02	7.00E+02	2.47E-02	2.63E-02	-1.58
TS-1'	<6.67E+00	4.45E-04	<6.67E+00	2.35E-04	3.40E-04	>-3.47
TS-3'						
TS-10'						
TS-30'						
TS-60'						
AVG VC	1.50E+04		2.84E+04			
VC-0'	3.00E+04		2.20E+04			
VC-60'	0.00E+00		3.47E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	1.12E+03	4.37E-02	1.00E+03	1.90E-02	3.13E-02	-1.50
TS-1'	<6.67E+00	2.60E-04	<6.67E+00	1.27E-04	1.93E-04	>-3.71
TS-3'						
TS-10'						
TS-30'						
TS-60'						
AVG VC	2.57E+04		5.27E+04			
VC-0'	5.13E+04		5.20E+04			
VC-60'	0.00E+00		5.33E+04			

Table A5. Inactivation of HAV, Polio 1 and Echo 1 by 1 mg/l Free Chlorine at pH 7.0 and 25 C.

AVG CL2 CONCENTRATION 0'= 1.0 mg/l
60'= 0.12 mg/l

VIRUS = HAV

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	<6.67E+00	1.61E-04	<6.67E+00	5.11E-04	3.36E-04	>-3.47
TS-1'						
TS-3'						
TS-10'						
TS-30'						
TS-60'						
AVG VC	4.16E+04		1.31E+04			
VC-0'	3.64E+04		1.49E+04			
VC-60'	4.67E+04		1.12E+04			

VIRUS= POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	2.80E+01	7.79E-04	1.68E+02	3.07E-04	5.43E-04	-3.27
TS-1'	<6.67E+00	1.86E-04	<6.67E+00	1.22E-05	9.89E-05	>-4.00
TS-3'						
TS-10'						
TS-30'						
TS-60'						
AVG VC	3.60E+04		5.48E+05			
VC-0'	5.60E+04		5.46E+05			
VC-60'	1.59E+04		5.49E+05			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	2.80E+02	1.26E-03	7.00E+02	2.00E-01	1.01E-01	-1.00
TS-1'	<6.67E+00	3.01E-05	<6.67E+00	1.91E-03	9.68E-04	>-3.01
TS-3'						
TS-10'						
TS-30'						
TS-60'						
AVG VC	2.22E+05		3.50E+03			
VC-0'	2.34E+04		3.36E+03			
VC-60'	4.20E+05		3.64E+03			

Table A6. Inactivation of HAV, Polio 1 and Echo 1 by 1 mg/l Free Chlorine at pH 9.5 and 25 C.

AVG CL2 CONCENTRATION 0'= 1.0 mg/l
60'= 0.34 mg/l

VIRUS = HAV

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	1.68E+04	2.61E-01	1.18E+04	1.77E-01	2.19E-01	-0.66
TS-1'	8.07E+02	1.26E-02	1.20E+03	1.80E-02	1.53E-02	-1.82
TS-3'	<6.67E+00	1.04E-04	<6.67E+00	1.00E-04	1.02E-04	>-3.99
TS-10'						
TS-30'						
TS-60'						
AVG VC	6.43E+04		6.67E+04			
VC-0'	7.33E+04		6.67E+04			
VC-60'	5.53E+04		6.67E+04			

VIRUS= POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	2.70E+04	4.91E-01	3.10E+04	5.23E-01	5.07E-01	-0.30
TS-1'	1.94E+04	3.53E-01	2.05E+04	3.46E-01	3.49E-01	-0.46
TS-3'	8.20E+02	1.49E-02	3.27E+03	5.51E-02	3.50E-02	-1.46
TS-10'	<6.67E+00	1.21E-04	<6.67E+00	1.12E-04	1.17E-04	>-3.93
TS-30'						
TS-60'						
AVG VC	5.50E+04		5.93E+04			
VC-0'	5.53E+04		6.33E+04			
VC-60'	5.47E+04		5.53E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	4.13E+04	8.26E-01	4.93E+04	1.66E+00	1.24E+00	0.09
TS-1'	6.90E+04	1.38E+00	9.73E+04	3.28E+00	2.33E+00	0.37
TS-3'	2.67E+03	5.34E-02	7.53E+04	2.54E+00	1.30E+00	0.11
TS-10'	5.00E+03	1.00E-01	2.04E+03	6.88E-02	8.44E-02	-1.07
TS-30'	<6.67E+00	1.33E-04	<6.67E+00	2.25E-04	1.79E-04	>-3.75
TS-60'						
AVG VC	5.00E+04		2.97E+04			
VC-0'	4.87E+04		3.53E+04			
VC-60'	5.13E+04		2.40E+04			

Table A7. Inactivation of HAV, Polio 1 and Echo 1 by 5 mg/l Free Chlorine at pH 4.5 and 5 C.

AVG CL2 CONCENTRATION 0'= 4.97 mg/l
30'= 4.1 mg/l

VIRUS = HAV

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	5.87E+05	2.24E+00	*TNTC	*TNTC	2.24E+00	0.35
TS-1'	2.67E+01	1.02E-04	4.67E+01	2.00E-04	1.51E-04	-3.82
TS-3'	< 6.67E+00	2.55E-05	< 6.67E+00	2.86E-05	2.70E-05	>-4.57
TS-10'						
TS-30'						
AVG VC	2.62E+05		2.34E+05			
VC-0'	2.80E+05		2.67E+05			
VC-30'	2.44E+05		2.00E+05			

VIRUS= POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	1.33E+01	6.54E-04	1.13E+02	7.22E-03	3.94E-03	-2.40
TS-1'	< 6.67E+00	3.28E-04	< 6.67E+00	4.26E-04	3.77E-04	>-3.42
TS-3'						
TS-10'						
TS-30'						
AVG VC	2.04E+04		1.57E+04			
VC-0'	2.00E+04		1.80E+04			
VC-30'	2.07E+04		1.33E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	< 6.67E+00	1.28E-04	6.00E+01	1.21E-03	6.68E-04	-3.18
TS-1'			< 6.67E+00	1.34E-04	6.71E-05	> -4.17
TS-3'						
TS-10'						
TS-30'						
AVG VC	5.20E+04		4.97E+04			
VC-0'	5.07E+04		5.07E+04			
VC-30'	5.33E+04		4.87E+04			

*Too Numerous To Count

Table A8. Inactivation of HAV, Polio 1 and Echo 1 by 5 mg/l Free Chlorine at pH 7.0 and 5 C.

AVG CL2 CONCENTRATION 0'= 5.1 mg/l
60'= 3.3 mg/l

VIRUS = HAV

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	3.40E+03	2.17E-02	5.00E+02	4.83E-03	1.32E-02	-1.88
TS-1'	2.67E+01	1.70E-04	< 6.67E+00	6.44E-05	1.17E-04	-3.93
TS-3'	< 6.67E+00	4.25E-05			2.12E-05	> -4.67
TS-10'						
TS-30'						
TS-60'						
AVG VC	1.57E+05		1.04E+05			
VC-0'	1.47E+05		1.07E+05			
VC-60'	1.67E+05		1.00E+05			

VIRUS= POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	8.73E+02	2.26E-02	2.60E+01	8.48E-04	1.17E-02	-1.93
TS-1'	2.00E+01	5.17E-04	< 6.67E+00	2.18E-04	3.68E-04	-3.43
TS-3'	< 6.67E+00	1.73E-04			8.63E-05	> -4.06
TS-10'						
TS-30'						
TS-60'						
AVG VC	3.87E+04		3.07E+04			
VC-0'	4.60E+04		2.53E+04			
VC-60'	3.13E+04		3.60E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	4.33E+02	9.77E-03	< 6.67E+00	1.35E-04	4.95E-03	-2.30
TS-1'	< 6.67E+00	1.51E-04			7.53E-05	> -4.12
TS-3'						
TS-10'						
TS-30'						
TS-60'						
AVG VC	4.43E+04		4.94E+04			
VC-0'	4.73E+04		4.80E+04			
VC-60'	4.13E+04		5.07E+04			

Table A9. Inactivation of HAV, Polio 1 and Echo 1 by 5 mg/l Free Chlorine at pH 9.5 and 5 C.

AVG CL2 CONCENTRATION 0'= 5.08 mg/l
60'= 2.63 mg/l

VIRUS = HAV

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	*TNTC	TNTC	2.13E+03	1.28E-02	6.40E-03	-2.19
TS-1'	4.73E+03	3.01E-02	2.67E+01	1.60E-04	1.51E-02	-1.82
TS-3'	< 6.67E+00	4.25E-05	< 6.67E+00	4.01E-05	4.13E-05	> -4.38
TS-10'						
TS-30'						
TS-60'						
AVG VC	1.57E+05		1.67E+05			
VC-0'	1.67E+05		1.60E+05			
VC-60'	1.47E+05		1.73E+05			

VIRUS= POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	5.87E+02	4.77E-02	2.00E+03	1.39E-01	9.35E-02	-1.03
TS-1'	3.47E+02	2.82E-02	1.87E+02	1.30E-02	2.06E-02	-1.69
TS-3'	1.20E+02	9.74E-03	< 6.67E+00	4.65E-04	5.10E-03	-2.29
TS-10'	< 6.67E+00	5.42E-04			2.71E-04	> -3.57
TS-30'						
TS-60'						
AVG VC	1.23E+04		1.44E+04			
VC-0'	1.53E+04		1.80E+04			
VC-60'	9.33E+03		1.07E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	5.80E+03	1.80E-01				-0.75
TS-1'	5.80E+03	1.80E-01				-0.75
TS-3'	4.60E+03	1.42E-01				-0.85
TS-10'	1.13E+03	3.50E-02				-1.46
TS-30'	< 6.67E+00	2.07E-04				> -3.69
TS-60'						
AVG VC	3.23E+04					
VC-0'	3.33E+04					
VC-60'	3.13E+04					

*Too Numerous To Count

Table A10. Inactivation of HAV, Polio 1 and Echo 1 by 5 mg/l Free Chlorine at pH 4.5 and 25 C.

AVG CL2 CONCENTRATION 0'= 5.2 mg/l
30'= 5.0 mg/l

VIRUS = HAV

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	2.00E+01	1.59E-03	4.33E+02	6.56E-03	4.07E-03	-2.39
TS-1'	< 6.67E+00	5.29E-04	< 6.67E+00	1.01E-04	3.15E-04	> -3.50
TS-3'						
TS-10'						
TS-30'						
AVG VC	1.26E+04		6.60E+04			
VC-0'	1.20E+04		8.13E+04			
VC-30'	1.32E+04		5.07E+04			

VIRUS= POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	< 6.67E+00	7.52E-04	< 6.67E+00	2.41E-04	4.97E-04	> -3.30
TS-1'						
TS-3'						
TS-10'						
TS-30'						
AVG VC	8.87E+03		2.77E+04			
VC-0'	9.00E+03		3.27E+04			
VC-30'	8.73E+03		2.27E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	< 6.67E+00	7.88E-04	< 6.67E+00	1.69E-03	1.24E-03	> -2.91
TS-1'						
TS-3'						
TS-10'						
TS-30'						
AVG VC	8.47E+03		3.95E+03			
VC-0'	8.13E+03		3.90E+03			
VC-30'	8.80E+03		4.00E+03			

Table A11. Inactivation of HAV, Polio 1 and Echo 1 by 5 mg/l Free Chlorine at pH 7.0 and 25 C.

AVG CL2 CONCENTRATION 0'= 5.05 mg/l
30'= 4.6 mg/l

VIRUS = HAV

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	< 6.67E+00	2.50E-03	< 6.67E+00	1.28E-04	1.31E-03	>-2.88
TS-1'						
TS-3'						
TS-10'						
TS-30'						
AVG VC	2.67E+03		5.23E+04			
VC-0'	2.67E+03		5.73E+04			
VC-30'	2.67E+03		4.73E+04			

VIRUS= POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	< 6.67E+00	1.26E-03	< 6.67E+00	2.57E-04	7.58E-04	>-3.12
TS-1'						
TS-3'						
TS-10'						
TS-30'						
AVG VC	5.30E+03		2.60E+04			
VC-0'	5.30E+03		3.00E+04			
VC-30'	5.30E+03		2.20E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	< 6.67E+00	6.20E-04	< 6.67E+00	1.39E-03	1.01E-03	>-3.00
TS-1'						
TS-3'						
TS-10'						
TS-30'						
AVG VC	1.08E+04		4.80E+03			
VC-0'	1.10E+04		4.80E+03			
VC-30'	1.05E+04		4.80E+03			

Table A12. Inactivation of HAV, Polio 1 and Echo 1 by 5 mg/l Free Chlorine at pH 9.5 and 25 C.

AVG CL2 CONCENTRATION 0'= 5.08 mg/l
30'= 4.8 mg/l

VIRUS = HAV

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	1.90E+02	9.05E-03	2.20E+02	3.79E-03	6.42E-03	-2.19
TS-1'	6.67E+00	3.18E-04	< 6.67E+00	1.15E-04	2.16E-04	>-3.66
TS-3'						
TS-10'						
TS-30'						
AVG VC	2.10E+04		5.80E+04			
VC-0'	2.13E+04		5.30E+04			
VC-30'	2.07E+04		6.30E+04			

VIRUS= POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	6.07E+02	3.64E-01	4.20E+03	1.60E-01	2.62E-01	-0.58
TS-1'	7.30E+01	4.38E-02	3.33E+01	1.27E-03	2.25E-02	-1.65
TS-3'	< 6.67E+00	4.00E-03	< 6.67E+00	2.54E-04	2.13E-03	>-2.67
TS-10'						
TS-30'						
AVG VC	1.67E+03		2.63E+04			
VC-0'	2.67E+03		3.33E+04			
VC-30'	6.67E+02		1.93E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	4.90E+04	4.43E+00	1.47E+04	3.37E+00	3.90E+00	0.59
TS-1'	2.90E+04	2.62E+00	4.27E+03	9.78E-01	1.80E+00	0.26
TS-3'	2.80E+03	2.53E-01	< 6.67E+00	1.53E-03	1.27E-01	-0.89
TS-10'	< 6.67E+00	6.04E-04			3.02E-04	> -3.52
TS-30'						
AVG VC	1.11E+04		4.37E+03			
VC-0'	1.01E+04		4.60E+03			
VC-30'	1.20E+04		4.13E+03			

Table A13. Inactivation of HAV, Strain MD-1 and Echo 1 by 1 mg/l Free Chlorine at pH 9.5 and 5 C.

AVG CL2 CONCENTRATION 0'= 1.06 mg/l
60'= 0.9 mg/l

VIRUS = HAV Strain MD-1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	8.67E+02	5.34E-01	2.30E+02	1.64E-01	3.49E-01	-0.46
TS-1'	1.93E+02	1.19E-01	1.20E+02	8.57E-02	1.02E-01	-0.99
TS-3'	< 6.67E+00	4.10E-03	< 6.67E+00	4.76E-03	4.43E-03	> -2.35
TS-10'						
TS-30'						
TS-60'						
AVG VC	1.63E+03		1.40E+03			
VC-0'	1.30E+03		9.60E+02			
VC-60'	1.95E+03		1.84E+03			

VIRUS= ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	5.13E+04	1.30E+00	3.80E+04	9.92E-01	1.15E+00	0.06
TS-1'	9.33E+04	2.36E+00	9.07E+04	2.37E+00	2.37E+00	0.37
TS-3'	3.50E+05	8.86E+00	3.60E+05	9.40E+00	9.13E+00	0.96
TS-10'	4.07E+05	1.03E+01	4.80E+05	1.25E+01	1.14E+01	1.06
TS-30'	1.60E+05	4.05E+00	1.39E+05	3.63E+00	3.84E+00	0.58
TS-60'	5.73E+04	1.45E+00	1.28E+04	3.34E-01	8.92E-01	-0.05
AVG VC	3.95E+04		3.83E+04			
VC-0'	2.50E+04		3.53E+04			
VC-60'	5.40E+04		4.13E+04			

TABLE A14. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY 1 TABLET/QUART IODINE AT pH 4.5 AND 5 C

AVERAGE I2 CONCENTRATION: 0' = 8.72 MG/L
60' = 7.13 MG/L

VIRUS = HEPATITIS A

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	3.47E+03	3.47E-01	1.80E+04	1.68E-01	2.58E-01	-0.59
TS-1'	1.60E+03	1.60E-01	1.73E+04	1.62E-01	1.61E-01	-0.79
TS-3'	1.20E+03	1.20E-01	1.47E+04	1.37E-01	1.29E-01	-0.89
TS-10'	6.80E+02	6.80E-02	3.07E+03	2.87E-02	4.83E-02	-1.32
TS-30'	< 6.67E+00	< 6.67E-04	< 6.67E+00	< 6.23E-05	< 3.65E-04	> -3.44
TS-60'						
VC-0'	1.00E+04		1.07E+05			
VC-60'	9.33E+03		1.00E+05			

VIRUS = POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	5.13E+03	7.69E-01	8.80E+03	6.29E-01	6.99E-01	-0.16
TS-1'	5.73E+03	8.59E-01	8.33E+03	5.95E-01	7.27E-01	-0.14
TS-3'	7.26E+03	1.09E+00	7.80E+03	5.57E-01	8.23E-01	-0.08
TS-10'	3.73E+03	5.59E-01	9.33E+03	6.66E-01	6.13E-01	-0.21
TS-30'	3.13E+03	4.69E-01	8.80E+03	6.29E-01	5.49E-01	-0.26
TS-60'	1.67E+03	2.50E-01	5.73E+03	4.09E-01	3.30E-01	-0.48
VC-0'	6.67E+03		1.40E+04			
VC-60'	6.13E+03		1.20E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	2.00E+03	1.54E-01	2.80E+03	2.48E-01	2.01E-01	-0.70
TS-1'	2.00E+03	1.54E-01	4.80E+03	4.25E-01	2.89E-01	-0.54
TS-3'	6.00E+03	4.62E-01	1.03E+04	9.12E-01	6.87E-01	-0.16
TS-10'	2.27E+04	1.75E+00	1.53E+04	1.35E+00	1.55E+00	0.19
TS-30'	2.60E+04	2.00E+00	1.30E+04	1.15E+00	1.58E+00	0.20
TS-60'	2.20E+02	1.69E-02	2.00E+02	1.77E-02	1.73E-02	-1.76
VC-0'	1.30E+04		1.13E+04			
VC-60'	2.33E+04		9.60E+03			

TABLE A15. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY 1 TABLET/QUART IODINE AT pH 7.0 AND 5 C

AVERAGE I2 CONCENTRATION: 0' = 8.58 MG/L
60' = 7.29 MG/L

VIRUS = HEPATITIS A

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	1.07E+03	1.65E-02	9.87E+03	1.23E-01	7.00E-02	-1.16
TS-1'	< 6.67E+00	< 1.03E-04	1.33E+02	1.66E-03	< 8.83E-04	> -3.05
TS-3'	< 6.67E+00	< 1.03E-04	< 6.67E+00	< 8.34E-05	< 9.32E-05	> -4.03
TS-10'						
TS-30'						
TS-60'						
VC-0'	6.47E+04		8.00E+04			
VC-60'	6.27E+04		4.40E+04			

VIRUS = POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	1.31E+04	3.71E-01	4.60E+03	5.75E-01	4.73E-01	-0.33
TS-1'	1.19E+04	3.37E-01	2.13E+03	2.66E-01	3.02E-01	-0.52
TS-3'	7.07E+03	2.00E-01	2.20E+03	2.75E-01	2.38E-01	-0.62
TS-10'	5.93E+03	1.68E-01	1.25E+03	1.56E-01	1.62E-01	-0.79
TS-30'	3.07E+03	8.70E-02	5.00E+02	6.25E-02	7.47E-02	-1.13
TS-60'	5.20E+02	1.47E-02	4.00E+01	5.00E-03	9.87E-03	-2.01
VC-0'	3.53E+04		8.00E+03			
VC-60'	2.29E+04		7.87E+03			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	1.03E+03	8.73E-02	8.00E+03	4.57E-01	2.72E-01	-0.57
TS-1'	4.53E+03	3.84E-01	1.14E+04	6.51E-01	5.18E-01	-0.29
TS-3'	1.18E+04	1.00E+00	5.60E+04	3.20E+00	2.10E+00	0.32
TS-10'	1.07E+02	9.07E-03	5.60E+04	3.20E+00	1.60E+00	0.21
TS-30'	< 6.67E+00	< 5.65E-04	< 6.67E+00	< 3.81E-04	< 4.73E-04	> -3.32
TS-60'						
VC-0'	1.18E+04		1.75E+04			
VC-60'	9.20E+03		1.49E+04			

TABLE A16. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY 1 TABLET/QUART IODINE AT pH 9.5 AND 5 C

AVERAGE I2 CONCENTRATION: 0' = 8.84 MG/L
30' = 6.30 MG/L

VIRUS = HEPATITIS A

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	< 6.67E+00	< 4.54E-04	< 6.67E+00	< 8.13E-05	< 2.68E-04	> -3.57
TS-1'						
TS-3'						
TS-10'						
TS-30'						
VC-0'	1.47E+04		8.40E+04			
VC-30	2.73E+04		8.20E+04 = VC-60			

VIRUS = POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	3.87E+03	8.80E-02	9.93E+02	2.61E-02	5.70E-02	-1.24
TS-1'	1.20E+02	2.73E-03	8.00E+01	2.11E-03	2.42E-03	-2.62
TS-3'	3.33E+01	7.57E-04	< 6.67E+00	< 1.76E-04	< 4.66E-04	> -3.33
TS-10'	< 6.67E+00	< 1.52E-04	< 6.67E+00	< 1.76E-04	< 1.64E-04	> -3.79
TS-30'						
VC-0'	4.40E+04		3.80E+04			
VC-30	7.20E+04		2.00E+04 = VC-60			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	1.67E+05	9.65E+00	1.09E+04	4.80E+00	7.23E+00	0.86
TS-1'	2.20E+03	1.27E-01	1.46E+03	6.43E-01	3.85E-01	-0.41
TS-3'	< 6.67E+00	< 3.86E-04	< 6.67E+00	< 2.94E-03	< 1.66E-03	> -2.78
TS-10'						
TS-30'						
VC-0'	1.73E+04		2.27E+03			
VC-30	3.67E+04		1.98E+03 = VC-60			

TABLE A17. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY 1 TABLET/QUART IODINE AT pH 4.5 AND 25 C

AVERAGE I2 CONCENTRATION: 0' = 8.03 MG/L
60' = 6.24 MG/L

VIRUS = HEPATITIS A

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	6.13E+02	6.61E-02	1.33E+03	1.32E-01	9.89E-02	-1.00
TS-1'	1.80E+03	1.94E-01	1.33E+03	1.32E-01	1.63E-01	-0.79
TS-3'	1.60E+02	1.73E-02	1.10E+02	1.09E-02	1.41E-02	-1.85
TS-10'	< 6.67E+00	< 7.20E-04	< 6.67E+00	< 6.60E-04	< 6.90E-04	> -3.16
TS-30'						
TS-60'						
VC-0'	9.27E+03		1.01E+04			
VC-60'	6.33E+03		1.02E+04			

VIRUS = POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	2.00E+03	2.38E-01	1.45E+04	3.69E-01	3.04E-01	-0.52
TS-1'	3.33E+03	3.96E-01	1.45E+04	3.69E-01	3.83E-01	-0.42
TS-3'	1.47E+03	1.75E-01	1.45E+04	3.69E-01	2.72E-01	-0.57
TS-10'	9.33E+02	1.11E-01	4.47E+03	1.14E-01	1.12E-01	-0.95
TS-30'	< 6.67E+00	< 7.94E-04	< 6.67E+00	< 1.70E-04	< 4.82E-04	> -3.32
TS-60'						
VC-0'	8.40E+03		3.93E+04			
VC-60'	7.00E+03		5.60E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	7.20E+03	8.37E-01	8.87E+03	2.29E-01	5.33E-01	-0.27
TS-1'	7.07E+03	8.22E-01	5.00E+04	1.29E+00	1.06E+00	0.02
TS-3'	6.00E+03	6.98E-01	5.47E+04	1.41E+00	1.06E+00	0.02
TS-10'	6.67E+00	7.76E-04	< 6.67E+00	< 1.72E-04	< 4.74E-04	> -3.32
TS-30'	< 6.67E+00	< 7.76E-04	< 6.67E+00	< 1.72E-04	< 4.74E-04	> -3.32
TS-60'						
VC-0'	8.60E+03		3.87E+04			
VC-60'	8.53E+03		1.01E+04			

TABLE A18. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY 1 TABLET/QUART IODINE AT pH 7.0 AND 25 C

AVERAGE I2 CONCENTRATION: 0' = 8.51 MG/L
60' = 7.71 MG/L

VIRUS = HEPATITIS A

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	< 6.67E+00	< 7.76E-04	< 6.67E+00	< 7.67E-05	< 4.26E-04	> -3.37
TS-1'						
TS-3'						
TS-10'						
TS-30'						
TS-60'						
VC-0'	8.60E+03		8.70E+04			
VC-60'	6.80E+03		9.60E+04			

VIRUS = POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	7.13E+03	7.75E-01	1.74E+04	1.18E+00	9.75E-01	-0.01
TS-1'	3.00E+03	3.26E-01	1.90E+04	1.28E+00	8.05E-01	-0.09
TS-3'	6.67E+00	7.25E-04	< 6.67E+00	< 4.51E-04	< 5.88E-04	> -3.23
TS-10'	< 6.67E+00	< 7.25E-04	< 6.67E+00	< 4.51E-04	< 5.88E-04	> -3.23
TS-30'						
TS-60'						
VC-0'	9.20E+03		1.48E+04			
VC-60'	8.93E+03		2.10E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	6.33E+03	6.93E-01	2.15E+03	1.95E+00	1.32E+00	0.12
TS-1'	< 6.67E+00	< 7.31E-04	1.80E+03	1.64E+00	< 8.19E-01	> -0.09
TS-3'	< 6.67E+00	< 7.31E-04	< 6.67E+00	< 6.06E-03	< 3.40E-03	> -2.47
TS-10'						
TS-30'						
TS-60'						
VC-0'	9.13E+03		1.10E+03			
VC-60'	8.73E+03		1.48E+03			

TABLE A19. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY 1 TABLET/QUART IODINE AT pH 9.5 AND 25 C

AVERAGE I2 CONCENTRATION: 0' = 8.00 MG/L
60' = 1.57 MG/L

VIRUS = HEPATITIS A

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	< 6.67E+00	< 2.67E-05	< 6.67E+00	< 5.85E-05	< 4.26E-05	> -4.37
TS-1'						
TS-3'						
TS-10'						
TS-30'						
TS-60'						
VC-0'	2.50E+05		1.14E+05			
VC-60'	2.47E+05		1.21E+05			

VIRUS = POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	3.53E+02	1.68E-02	9.00E+01	3.33E-03	1.01E-02	-2.00
TS-1'	5.33E+01	2.54E-03	< 6.67E+00	< 2.47E-04	< 1.39E-03	> -2.86
TS-3'	2.00E+01	9.52E-04	< 6.67E+00	< 2.47E-04	< 6.00E-04	> -3.22
TS-10'	< 6.67E+00	< 3.18E-04	< 6.67E+00	< 2.47E-04	< 2.82E-04	> -3.55
TS-30'						
TS-60'						
VC-0'	2.10E+04		2.70E+04			
VC-60'	1.14E+04		3.10E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	5.47E+03	6.84E-01	3.30E+03	3.03E+00	1.86E+00	0.27
TS-1'	< 6.67E+00	< 8.34E-04	< 6.67E+00	< 6.12E-03	< 3.48E-03	> -2.46
TS-3'						
TS-10'						
TS-30'						
TS-60'						
VC-0'	8.00E+03		1.09E+03			
VC-60'	1.27E+04		5.10E+03			

TABLE A20. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY 2 TABLETS/QUART IODINE AT pH 4.5 AND 5 C

AVERAGE I2 CONCENTRATION: 0' = 17.63 MG/L
30' = 16.70 MG/L
60' = 16.46 MG/L

VIRUS = HEPATITIS A

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	2.73E+03	1.86E-01	4.80E+03	3.36E-01	2.61E-01	-0.58
TS-1'	3.00E+03	2.04E-01	3.53E+03	2.47E-01	2.25E-01	-0.65
TS-3'	1.25E+03	8.50E-02	1.53E+03	1.07E-01	9.60E-02	-1.02
TS-10'	1.13E+03	7.69E-02	5.87E+02	4.10E-02	5.90E-02	-1.23
TS-30'	1.70E+01	1.16E-03	1.53E+02	1.07E-02	5.93E-03	-2.23
TS-60'	1.33E+00	9.05E-05	2.00E+00	1.40E-04	1.15E-04	-3.94
VC-0'	1.47E+04		1.43E+04			
VC-60'	1.29E+04		1.50E+04			

VIRUS = POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	1.93E+04	2.14E-01	2.47E+04	2.35E-01	2.25E-01	-0.65
TS-1'	2.27E+04	2.52E-01	3.40E+04	3.24E-01	2.88E-01	-0.54
TS-3'	1.63E+04	1.81E-01	2.93E+04	2.79E-01	2.30E-01	-0.64
TS-10'	1.28E+04	1.42E-01	2.13E+04	2.03E-01	1.73E-01	-0.76
TS-30'	7.80E+03	8.67E-02	1.08E+04	1.03E-01	9.48E-02	-1.02
TS-60'	1.40E+03	1.56E-02	1930.00	1.84E-02	1.70E-02	-1.77
VC-0'	9.00E+04		1.05E+05			
VC-60'	9.07E+04		9.93E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	5.60E+03	5.00E-02	9.33E+03	9.15E-02	7.07E-02	-1.15
TS-1'	2.00E+04	1.79E-01	3.53E+04	3.46E-01	2.62E-01	-0.58
TS-3'	7.07E+04	6.31E-01	9.33E+04	9.15E-01	7.73E-01	-0.11
TS-10'	2.20E+05	1.96E+00	1.00E+05	9.80E-01	1.47E+00	0.17
TS-30'	3.53E+03	3.15E-02	2.47E+03	2.42E-02	2.79E-02	-1.55
TS-60'	6.67E+00	5.96E-05	6.67E+00	6.54E-05	6.25E-05	-4.20
VC-0'	1.12E+05		1.02E+05			
VC-60'	4.27E+04		1.17E+05			

TABLE A21. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY 2 TABLETS/QUART IODINE AT pH 7.0 AND 5 C

AVERAGE I2 CONCENTRATION: 0' = 17.74 MG/L
60' = 16.25 MG/L

VIRUS = HEPATITIS A

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	8.20E+02	1.58E-02	1.00E+05	2.78E+00	1.40E+00	0.15
TS-1'	< 6.67E+00	< 1.28E-04	< 6.67E+00	< 1.85E-04	< 1.57E-04	> -3.80
TS-3'						
TS-10'						
TS-30'						
TS-60'						
VC-0'	5.20E+04		3.60E+04			
VC-60'	7.00E+04		4.13E+04			

VIRUS = POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	2.67E+03	4.17E-01	1.36E+04	2.22E-01	3.20E-01	-0.50
TS-1'	2.20E+03	3.44E-01	1.35E+04	2.20E-01	2.82E-01	-0.55
TS-3'	2.20E+03	3.44E-01	1.31E+04	2.14E-01	2.79E-01	-0.55
TS-10'	1.47E+03	2.30E-01	1.39E+04	2.27E-01	2.28E-01	-0.64
TS-30'	1.27E+02	1.98E-02	2.73E+03	4.45E-02	3.22E-02	-1.49
TS-60'	< 6.67E+00	< 1.04E-03	< 6.67E+00	< 1.09E-04	< 5.75E-04	> -3.24
VC-0'	6.40E+03		6.13E+04			
VC-60'	7.80E+03		7.67E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	2.33E+04	5.72E-01	6.40E+03	9.85E-02	3.35E-01	-0.47
TS-1'	5.00E+04	1.23E+00	4.20E+04	6.46E-01	9.37E-01	-0.03
TS-3'	7.53E+03	1.85E-01	3.93E+04	6.05E-01	3.95E-01	-0.40
TS-10'	< 6.67E+00	< 1.64E-04	4.00E+01	6.15E-04	< 3.90E-04	> -3.41
TS-30'	< 6.67E+00	< 1.64E-04	< 6.67E+00	< 1.03E-04	< 1.33E-04	> -3.88
TS-60'						
VC-0'	4.07E+04		6.50E+04			
VC-60'	6.80E+04		7.20E+04			

TABLE A22. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY 2 TABLETS/QUART IODINE AT pH 9.5 AND 5 C

AVERAGE I2 CONCENTRATION: 0' = 16.01 MG/L
30' = 13.43 MG/L

VIRUS = HEPATITIS A

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	< 6.67E+00	< 1.32E-04	< 6.67E+00	< 1.64E-04	< 1.48E-04	> -3.83
TS-1'						
TS-3'						
TS-10'						
TS-30'						
VC-0'	5.07E+04		4.07E+04			
VC-30	5.40E+04		4.47E+04			

VIRUS = POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	8.33E+03	7.24E-02	6.93E+03	5.54E-02	6.39E-02	-1.19
TS-1'	4.67E+02	4.06E-03	4.07E+02	3.26E-03	3.66E-03	-2.44
TS-3'	8.67E+01	7.54E-04	6.67E+01	5.34E-04	6.44E-04	-3.19
TS-10'	2.67E+01	2.32E-04	2.67E+01	2.14E-04	2.23E-04	-3.65
TS-30'	< 6.67E+00	< 5.80E-05	< 6.67E+00	< 5.34E-05	< 5.57E-05	> -4.25
VC-0'	1.15E+05		1.25E+05			
VC-30	1.16E+05		1.15E+05			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	1.80E+05	1.43E+00	1.73E+05	1.92E+00	1.68E+00	0.22
TS-1'	3.07E+03	2.44E-02	5.20E+02	5.78E-03	1.51E-02	-1.82
TS-3'	4.00E+01	3.17E-04	< 6.67E+00	< 7.41E-05	< 1.96E-04	> -3.71
TS-10'	1.33E+01	1.06E-04	< 6.67E+00	< 7.41E-05	< 8.98E-05	> -4.05
TS-30'	< 6.67E+00	< 5.29E-05	< 6.67E+00	< 7.41E-05	< 6.35E-05	> -4.20
VC-0'	1.26E+05		9.00E+04			
VC-30	1.30E+05		9.40E+04			

TABLE A23. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY 2 TABLETS/QUART IODINE AT pH 4.5 AND 25 C

AVERAGE I2 CONCENTRATION: 0' = 17.18 MG/L
30' = 15.16 MG/L

VIRUS = HEPATITIS A

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	4.13E+03	2.70E-01	3.60E+03	2.25E-01	2.47E-01	-0.61
TS-1'	1.47E+03	9.61E-02	2.60E+03	1.63E-01	1.29E-01	-0.89
TS-3'	2.67E+02	1.75E-02	4.67E+02	2.92E-02	2.33E-02	-1.63
TS-10'	< 6.67E+00	< 4.36E-04	< 6.67E+00	< 4.17E-04	< 4.26E-04	> -3.37
TS-30'						
VC-0'	1.53E+04		1.60E+04			
VC-30	2.07E+04		2.06E+04			

VIRUS = POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	5.73E+03	4.58E-01	6.67E+03	6.29E-01	5.44E-01	-0.26
TS-1'	5.07E+03	4.06E-01	8.80E+03	8.30E-01	6.18E-01	-0.21
TS-3'	2.80E+03	2.24E-01	4.73E+03	4.46E-01	3.35E-01	-0.47
TS-10'	< 6.67E+00	< 5.34E-04	6.67E+00	6.29E-04	< 5.81E-04	> -3.24
TS-30'	< 6.67E+00	< 5.34E-04	< 6.67E+00	< 6.29E-04	< 5.81E-04	> -3.24
VC-0'	1.25E+04		1.06E+04			
VC-30	1.23E+04		1.45E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	1.19E+04	1.19E+00	1.05E+04	7.50E-01	9.70E-01	-0.01
TS-1'	4.13E+04	4.13E+00	4.80E+04	3.43E+00	3.78E+00	0.58
TS-3'	1.30E+02	1.30E-02	3.33E+02	2.38E-02	1.84E-02	-1.74
TS-10'	< 6.67E+00	< 6.67E-04	1.33E+01	9.50E-04	< 8.09E-04	> -3.09
TS-30'	< 6.67E+00	< 6.67E-04	< 6.67E+00	< 4.76E-04	< 5.72E-04	> -3.24
VC-0'	1.00E+04		1.40E+04			
VC-30	1.10E+04		1.40E+04			

TABLE A24. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY 2 TABLETS/QUART IODINE AT pH 7.0 AND 25 C

AVERAGE I2 CONCENTRATION: 0' = 16.51 MG/L
30' = 13.21 MG/L

VIRUS = HEPATITIS A

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	< 6.67E+00	< 2.13E-04	< 6.67E+00	< 7.41E-04	< 4.77E-04	> -3.32
TS-1'						
TS-3'						
TS-10'						
TS-30'						
VC-0'	3.13E+04		9.00E+03			
VC-30	3.07E+04		1.40E+04			

VIRUS = POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	4.40E+03	1.57E-01	8.80E+02	1.65E-01	1.61E-01	-0.79
TS-1'	2.53E+03	9.04E-02	3.07E+02	5.76E-02	7.40E-02	-1.13
TS-3'	2.00E+01	7.14E-04	6.67E+00	1.25E-03	9.83E-04	-3.01
TS-10'	< 6.67E+00	< 2.38E-04	< 6.67E+00	< 1.25E-03	< 7.45E-04	> -3.13
TS-30'						
VC-0'	2.80E+04		5.33E+03			
VC-30	2.33E+04		5.86E+03			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	1.01E+05	4.21E+00	1.70E+04	7.52E-01	2.48E+00	0.39
TS-1'	1.30E+01	5.42E-04	< 6.67E+00	< 2.95E-04	< 4.18E-04	> -3.38
TS-3'	< 6.67E+00	< 2.78E-04	< 6.67E+00	< 2.95E-04	< 2.87E-04	> -3.54
TS-10'						
TS-30'						
VC-0'	2.40E+04		2.26E+04			
VC-30	2.80E+04		2.80E+04			

TABLE A25. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY 2 TABLETS/QUART IODINE AT pH 9.5 AND 25 C

AVERAGE I2 CONCENTRATION: 0' = 16.52 MG/L
30' = 2.20 MG/L

VIRUS = HEPATITIS A

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	< 6.67E+00	< 2.64E-04	< 6.67E+00	< 2.22E-04	< 2.43E-04	> -3.61
TS-1'						
TS-3'						
TS-10'						
TS-30'						
VC-0'	2.53E+04		3.00E+04			
VC-30	2.26E+04		4.67E+04			

VIRUS = POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	< 6.67E+00	< 6.72E-04	6.67E+00	1.57E-03	< 1.12E-03	> -2.95
TS-1'	< 6.67E+00	< 6.72E-04	< 6.67E+00	< 1.57E-03	< 1.12E-03	> -2.95
TS-3'						
TS-10'						
TS-30'						
VC-0'	9.93E+03		4.26E+03			
VC-30	9.93E+03		3.33E+03			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	2.40E+02	3.43E-03	3.47E+02	5.10E-03	4.27E-03	-2.37
TS-1'	< 6.67E+00	< 9.53E-05	< 6.67E+00	< 9.81E-05	< 9.67E-05	> -4.01
TS-3'						
TS-10'						
TS-30'						
VC-0'	7.00E+04		6.80E+04			
VC-30	7.00E+04		6.40E+04			

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